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Merkel Cell Carcinoma

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Highlights

Staphylococcus Epidermidis

Antibiotics in Staphylococcus

Discovering Thoughts, Inventing Future

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Evaluation of Sensitivity of Commonly used Antibiotics in Staphylococcus Epidermidis Clinical Isolates From Assir Region, Saudi Arabia

By Nazar Mohamed Abdalla, Waleed Omer Haimour, Amani Ali Osman, Faten Mohamed ElAbd, Hassan Abdulaziz Musa & Mohamed Nazar Mohamed
Medicine/ Gezira University, Saudi Arabia

Abstract- Background: Multidrugs resistance is an emerging health problem that ultimately will lead to vanishing of effective medicine against infections including Staphylococcus epidermidis infections.

Aim: This a prospective hospital base study of 58 Staphylococcus epidermidis clinical isolates in Assir region aim at evaluating the sensitivity profile of commonly used antibiotic during the period of March 2011- Sep. 2011.

Materials and Methods: Bacteriology procedures ; staining, culture, catalase, coagulase and antibiotics sensitivity test using diffusion disc test, minimum inhibitory concentration (MIC) and molecular (PCR) for confirmation of Staphylococcal species and detection of the Mec A gene.

Keywords: staphylococcus epidermidis, coagulasenegative staphylococci (CoNS), antimicrobial resistance (AMR), nosocomial infections, diabetes.

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Nazar Mohamed Abdalla [α], Waleed Omer Haimour [σ], Amani Ali Osman [ρ], Faten Mohamed ElAbd [ω], Hassan Abdulaziz Musa [¥] & Mohamed Nazar Mohamed [§]

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Result: 58 Staphylococcus epidermidis clinical isolates including 14 diabetics. Age groups include 29 (0-15yrs), 14 (16-50yrs) and 15 (50yrs& above). The total resistance cases to Oxacillin/ Mithicillin was found to be 56 cases (96.4%); all non diabetics were resistance. Resistance and sensitivity to Ciprofloxacin among diabetic and non diabetic were 75.9% and 24.1% respectively. Total resistance to Fusidin were 81%, while total resistant to Erythromycin in all ages groups were 86.2%. In age group (0-15) years 93.1% were resistant to the drug which comprises, 54% of the total resistant cases (n=50) and 46.6% from all Staphylococcus epidermidis cases (n=58).

Conclusion: Staphylococcus epidermidis is a pathogen associated with community acquired and nosocomial infections. The nosocomial infections are predominant in neonatal intensive care units (NICU). Resistance of Erythromycin in S. epidermidis cases among children is highly observed as this drug is commonly used by this age group. Diabetes has equivocal effect on drugs sensitivity. The frequency of staphylococcus multi-drugs resistance is rising.

Author α: Consultant Medical Microbiologist, College of Medicine, King Khalid University, Abha, Saudi Arabia. e-mail: uofgnazar@gmail.com
Author σ ω: Microbiology Specialist, Assir Central Hospital Lab, Abha, Saudi Arabia.
Author ρ: Consultant Reproductive Health, Family & Community Medicine Department, College of Medicine, King Khalid University, Abha, Saudi Arabia.
Author ¥: Consultant Microbiologist, The Vice Chancellor's Assistant for Academic Affairs, The National Ribat University, Khartoum, Sudan.
Author §: Medical Officer, Khartoum Hospital, Ministry of Health, Sudan.

Keywords: staphylococcus epidermidis, coagulase-negative staphylococci (CoNS), antimicrobial resistance (AMR), nosocomial infections, diabetes.

I. Introduction

Friedrich Julius Rosenbach distinguished S. *epidermidis* from S. *aureus* in 1884, initially naming S. *epidermidis* as S. *albus.* He chose *aureus* and *albus* since the bacteria formed yellow and white colonies, respectively. S. *epidermidis* causing nosocomial and community acquired infections [1]

S. *epidermidis* is a very hardy microorganism, consisting of nonmotile, Gram-positive cocci, arranged in grape-like clusters. It forms white, raised colonies approximately 1–2 millimeter in diameter after overnight incubation, and is nonhemolytic on blood agar. It is a catalase-positive, coagulase-negative, facultative anaerobe that can grow by aerobic respiration or by fermentation. Some strains may not ferment [2].

Biochemical tests indicate this microorganism also carries out a weakly positive reaction to the nitrate reductase test. It is positive for urease production, is oxidase negative, and can use glucose, sucrose, and lactose to form acid products. In the presence of lactose, it will also produce gas. *S. epidermidis* does not possess the gelatinase enzyme, so it cannot hydrolyze gelatin. It is sensitive to novobiocin, providing an important test to distinguish it from *Staphylococcus saprophyticus*, which is coagulase-negative, as well, but novobiocin-resistant. Similar to those of *Staphylococcus aureus*, the cell walls of *S. epidermidis* have a transferrin binding protein that helps the organism obtain iron from transferrin. The tetramers of a surface exposed protein, glyceraldehyde-3-phosphate dehydrogenase, are believed to bind to transferrin and remove its iron. Subsequent steps include iron being transferred to surface lipoproteins, then to transport proteins which carry the iron into the cell [3] [3]. The normal practice of detecting *S. epidermidis* is by using the Baird-Parker agar with egg yolk supplement. Colonies appear small and black. They can be confirmed using the coagulase test. Increasingly, techniques such as real-time PCR and

quantitative PCR are being employed for the rapid detection and identification of *Staphylococcus* strains [4][4]. Normally, sensitivity to desferrioxamine can also be used to distinguish it from most other staphylococci, except in the case of *Staphylococcus hominis*, which is also sensitive. In this case, the production of acid from trehalose by *S. hominis* can be used to tell the two species apart.

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem. Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately.

Aseer Central Hospital is almost 600 bedded and it is accredited from The Central Board of Arab Health. It's laboratory is a regional referral hub. The other hand, the hospital is affiliated to the medical college of king Khalid University. This study aimed at evaluating the commonly used antibiotics resistant and the factors affecting the drugs sensitivity of Staphyloccocus epidermidis isolates from nasal swabs of patients presented at Aseer Central Hospital General Lab.

II. Material and Methods

The patients in this study were informed about the study content and procedures with preservation of human rights in concordance with the research ethics of the Deanship of Scientific Research and Research Center For Medical *College,* King Khalid University, Kingdom of Saudi Arabia.

A total of 58 clinical isolates including; respiratory infection, central nervous system infections, urogenital infection, musculoskeletal (Joints) infections and skin infection were included. Blood, urine and swabs (nasal, skin and conjunctivae) specimens have been tested by bacteriology, chemical and PCR Assay. Bacteriology procedures ; staining, culture, catalase, coagulase and antibiotics sensitivity test using diffusion disc test, minimum inhibitory concentration (MIC) [5] and molecular (PCR) for confirmation of Staphylococcal species [6] and detection of the Mec A gene [7]. General primers for detection of positive Staphylococcal isolates not carrying the Mc Agene were used. The codes and sequences of the primers (50 pmol of primer per reaction) were as follows: ERIC-1R, 59-ATG TAA GCT CCT GGG GAT TCA C-39; ERIC-2, 59-AAG TAA GTG ACT GGG GTG AGC G-39; (*Staphylococcus epidermidis* ATCC 12228 chromosome, complete genome NCBI Reference Sequence: NC_004461.1). The PCR mixture was overlaid with 5 ul of mineral oil to prevent evaporation. Amplification of DNA fragments was performed in a Biomed thermo-cycler (model 60; Biomed, Theres, Germany) with predenaturation at 94C° for 4 min, followed by 40 cycles of 1 min at 94C°, 1 min at 55C°, and 2 min at 74 C°. Amplicons were analyzed by agarose gel electrophoresis containing 1% agarose (Hispanagar; Sph. Leiden, The Netherlands) in 0.53 Tris-borate-EDTA (TBE) in the presence of ethidium bromide (0. 0.3 mg/ml) at a constant current of 100 mA for 1 h.

a) Statistical Study

Clinical and Laboratory data were recorded in special formats and entered in stat computer program (SPSS). Descriptive and analytical statistical analysis were performed and final results were plotted in tables.

III. Results

58 *Staphylococcus epidermidis* and negative Mec A gene clinical isolates including 14 diabetics. Age groups include 29 (0-15yrs), 14 (16-50yrs) and 15 (50yrs& above). 29 patients (50%) have presented with skin sepsis this due to the fact that *S. epidermidis* is a known normal flors of the skin. Distribution of patients according to their sex and diagnosis . Table 1.

Distribution of patients according to their presence in hospital revealed that; 35 patients were in intensive care units and 24 patients were in PICU and NICU (Pediatric and Neonates). Table 2.

The total resistance cases to Oxacillin/ Mithicillin was found to be 56 cases (96.4%); 12 diabetic patients (21.4%) and 44 non diabetic (78.6%). So all non diabetics were resistance. Table 3.

Resistance and sensitivity to Ciprofloxacin in all 58 *Staphylococcus epidermidis* diabetic and non diabetic patients under study were 75.9% and 24.1% respectively. Table 4.

Total resistance to Fusidin were 47 cases (81%) and total sensitivity to Fusidin were 11 cases (19%). Table 5

Total resistant and sensitivity to Erythromycin in all ages groups were 86.2% and 13.8% respectively. In age group (0-15) years 93.1% were resistant to the drug which comprises, 54% of the total resistant cases (n=50) and 46.6% from all *Staphylococcus epidermidis* cases (n=58). Table 6.

Table 1 : Distribution of patients according to their sex and diagnosis

Diagnosis	Sex		Total
	Male	Female	
Acute Abdomen	0	1	1
Sepsis	**18**	**11**	**29**
URI	2	0	2
Post Surgery	0	1	1
CVA	4	3	7
ESRD	1	0	1
RDS	1	0	1
PUO(Pyrexia)	1	0	1
ELEC Burn	1	0	1
Trauma	1	1	2
RTA	7	0	7
Head Inj.	1	1	2
DM	2	1	3
Total	39	19	58

Table 2 : Distribution of patients according to age group in different departments

Department	Age			Total
	(0- 15)years	(16 – 50) years	51 years and more	
OPD	2	4	2	8
MMW	0	0	1	1
MFSW	1	0	0	1
MSW	1	0	2	3
FMW	0	1	2	3
PMW	1	0	0	1
ER	1	1	0	2
PICU	**15**	**1**	**0**	**16**
MOW	0	0	1	1
ICU	0	2	2	4
IMCU	0	2	4	6
CCU	0	0	1	1
NICU	**8**	**0**	**0**	**8**
BU	0	1	0	1
MNW	0	1	0	1
MUW	0	1	0	1
Total	29	14	15	58

Table 3 : Crosstabs showed Oxacillin/ Mithicillin sensitivity and resistance in *Staphylococcus epidermidis* positive cases among diabetics and non diabetics

Antibiotic sensitivity profile			Diabetes mellitus		Total
			Yes	No	Yes
Oxacillin/ Mithicillin	R	Count	12	44	56
		% within Oxacillin/ Mithicillin	21,4%	78,6%	100,0%
		% within Diabetes mellitus	85,7%	100,0%	96,6%
		% of Total	20,7%	75,9%	96,6%
	S	Count	2	0	2
		% within Oxacillin/ Mithicillin	100,0%	,0%	100,0%
		% within Diabetes mellitus	14,3%	,0%	3,4%
		% of Total	3,4%	,0%	3,4%
Total		Count	14	44	58
		% within Oxacillin/ Mithicillin	24,1%	75,9%	100,0%
		% within Diabetes mellitus	100,0%	100,0%	100,0%
		% of Total	24,1%	75,9%	100,0%

Table 4 : Crosstabs showed Ciprofloxacin sensitivity and resistance in *Staphylococcus epidermidis* positive cases among diabetics and non diabetics

Antibiotic sensitivity profile			Diabetes mellitus		Total
			Yes	No	Yes
Ciprofloxacin	R	Count	10	34	44
		% within Ciprofloxacin	19,5%	80,5%	100,0%
		% within Diabetes mellitus	57,1%	75,0%	70,7%
		% of Total	13,8%	56,9%	70,7%
	S	Count	4	10	14
		% within Ciprofloxacin	28,6%	71,4%	100,0%
		% within Diabetes mellitus % of Total	28,6% 6,9%	22,7% 17,2%	24,1% 24,1%
	Total		14 24.1%	44 75.9%	58 100%

Table 5 : Crosstabs showed Fusidin sensitivity and resistance in *Staphylococcus epidermidis* positive cases

Antibiotic sensitivity profile			Diabetes mellitus		Total
			yes	no	
Fusidin	R	Count	11	36	47
		% within Fusidin	18,5%	81,5%	100,0%
		% within Diabetes mellitus	78.5%	81.8%	81%
		% of Total	18.9%	62.1%	81%
	S	Count	3	8	11
		% within Fusidin	27,3%	72,7%	100,0%
		% within Diabetes mellitus	21,4%	18,2%	19,0%
		% of Total	5,2%	13,8%	19,0%
	Total		14 24.1%	44 75.9%	58 100%

Table 6 : Crosstabs showed Erythromycin sensitivity and resistance in *Staphylococcus epidermidis* positive cases among different age groups

Antibiotic sensitivity profile			age group			Total
			0-15	16-50	51+	
Erythromycin	R	Count	27	11	12	50
		% within Erythromycin	54 %	22 %	24 %	100,0%
		% within age group	93,1%	78,6%	80 %	84,5%
		% of Total	46,6%	19,0%	19,0%	86,2%
	S	Count	2	3	3	8
		% within Erythromycin	25,0%	37,5%	37,5%	100,0%
		% within age group	6,9%	21,4%	20,0%	13,8%
		% of Total	3,4%	5,2%	5,2%	13,8%
Total		Count	29	14	15	58
		% within Erythromycin	50,0%	24,1%	25,9%	100,0%
		% within age group	100,0%	100,0%	100,0%	100,0%
		% of Total	50,0%	24,1%	25,9%	100,0%

IV. Discussion

Staphylococcus epidermidis is one of 33 known species belonging to the genus *Staphylococcus*. The taxonomy of this bacteria is; Kingdom: Bacteria. Phylum: Firmicutes. Class: Cocci. Oreder: Bacillales. Family: Staphylococcaceae. Genus: Satphylococcus. Species: S. epidermidis. It is part of human skin flora (commensal), and consequently part of human flora. It can also be found in the mucous membranes and in animals. Due to contamination, it is probably the most common species found in laboratory tests [8][7]. Although *S. epidermidis* is not usually pathogenic, patients with compromised immune systems are often at risk for developing an infection. These infections can be both nosocomial or community acquired, *S. epidermidis* is also a major concern for people with catheters or other surgical implants because it is known to cause biofilms that grow on these devices [9][8]. *S. epidermidis* causes biofilms to grow on plastic devices placed within the body [10][9]. This occurs most commonly on intravenous catheters and on medical prostheses. Infection can also occur in dialysis patients or anyone with an implanted plastic device that may have been contaminated. Another disease it causes endocarditis [11]. In some other cases, sepsis can occur in hospital patients. Resistant organisms are most commonly found in the intestine, but organisms living freely on the skin can also become resistant due to routine exposure to antibiotics secreted in sweat [12][12]. Detection of the mecA gene by polymerase chain reaction (PCR) is the gold standard for identifying methicillin-resistant Staphylococcus aureus (MRSA). PCR assays, employing MR1-MR2 primers (primer set 1) and MR3-MR4 primers (primer set 2) to generate 154 and 533 bp fragment, respectively, are most widely used for amplification of mecA gene [13][13].Spread of *S.* spp. (including MRSA) generally is through human-to-human contact, although recently some veterinarians have discovered the infection can be spread through pets, with environmental contamination. Cases of *S.* spp. Nosocomial infections have reported to be transported by polyester, the main material used in hospital curtains in hospitals across America [14][14]. An important and previously unrecognized means of community-associated MRSA colonization and transmission is during sexual contact [15][15]. It was discovered that there are two different strains of S. epidermidis, one that inhibits biofilm formation by S. aureus, S. epidermidis strain JK16 (inhibitory type), and one that does not (non-inhibitory type) S. epidermidis strain JK11 [16][16]. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme that cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. Penicillinase-resistant β-lactam antibiotics, such as methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, and flucloxacillin, are able to resist degradation by staphylococcal penicillinase. Resistance to methicillin is mediated via the *mec* operon, part of the staphylococcal cassette chromosome mec (SCC*mec*). Resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') that has a lower affinity for binding β-lactams (penicillins, cephalosporins, and carbapenems). This allows for resistance to all β-lactam antibiotics, and obviates their clinical use during MRSA infections. As such, the glycopeptide vancomycin is often deployed against MRSA [17][17]. Aminoglycoside antibiotics, such as kanamycin, gentamicin, streptomycin, etc., were once effective against staphylococcal infections until strains

evolved mechanisms to inhibit the aminoglycosides' action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosomal subunit [18][18]. There are three main mechanisms of aminoglycoside resistance mechanisms which are currently and widely accepted: aminoglycoside modifying enzymes, ribosomal mutations, and active efflux of the drug out of the bacteria [19][19]. MRSA infections in both the hospital and community setting are commonly treated with non-β-lactam antibiotics, such as clindamycin (a lincosamine) and co-trimoxazole (also commonly known as trimethoprim/ sulfamethoxazole). Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram-positive antibiotics, such as linezolid, because of its availability as an oral drug. So it is nowadays highly recommended to use combined therapy to treat severe cases of S. aureus infections such as pneumonia, meningitis and toxic shock syndrome [20].[20].All 29 S. epidermidis isolates were found to be resistant to oxacillin and were positive for the mecA gene. The isolates showed several multidrug-resistance patterns; the resistance rates to gentamicin, erythromycin, clindamycin, and [21]were susceptible to vancomycin, teicoplanin, rifampin, synercid, and ciprofloxacin. Several genotypic and phenotypic patterns were detected among the S. epidermidis isolates: antibiogram typing showed seven different patterns, one of which was shared by 65% of the isolates, whereas the most prevalent RAPD genotype was shared by only five S. epidermidis isolates [22], and did not correlate with antibiotic resistance phenotype. The diverse clonal origin of tested isolates indicates the presence of multiple S. epidermidis strains among neonates in the NICU setting [23][21]. In another study the nasal carriage of methicillin-resistant coagulase-negative staphylococci (MR-CoNS) is highly prevalent in community subjects [24][22]. Few studies on staphylococcal infections and drugs sensitivity were conducted in Saudi Arabia [25] [26] [27][24, 25, 26]. Resistance is conferred by Penicillinase-resistant β-lactam antibiotics and the mec A gene, which codes for an altered penicillin-binding protein (PBP') that has a lower affinity for binding β-lactams (penicillins, cephalosporins, and carbapenems). This allows for resistance to all β-lactam antibiotics, and obviates their clinical use during MRSA infections. Mec A gene is known associated factor of drug resistance for Oxacillin/Mithcillin drug as all isolates were Mec A gene negative, the resistance could be explained by the thick biofilm caused by this bacteria which guard against drug penetration [28] [29],

V. Acknowledgement

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VI. Conclusion

Staphylococcus epidermidis is a pathogen associated with community acquired and nosocomial infections. These infections were predominant among children in neonatal intensive care units (NICU) .

Resistance of Erythromycin in *S. epidermidis* cases among children is highly observed as this drug is commonly used by this age group.

Diabetes has equivocal effect on drugs sensitivity. The frequency of staphylococcus multi-drugs resistance is rising as well in Asser region), involving variable drugs mode of actions; cell wall inhibitors, protein synthesis inhibitors and DNA gyrase inhibitors.

Rising of multidrug resistance could be attributed to genetic clone and the adherence of the pathogen to devices like ventilators and catheters .

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Merkel Cell Carcinoma : An Indian Experience

By Dr. Biswanath Paul, Dr. Bhaskar Mitra, Dr. Mallika Pal,
Dr. Tarak Nath Saha & Dr. Ashok Maiti

Midnapore Medical College & Hospital, India

Abstract- Merkel cell carcinoma [MCC] is a rare neoplasm of skin with an aggressive behaviour and unfavourable prognosis. MCC mainly occurs in the elderly, in the sun exposed areas of skin. Indian's experiences regarding patients' profiles, clinical presentation, age of occurrence, pattern of tumour are very limited as well as challenging.

This retrospective study was performed, in a tertiary care hospital in Paschim Medinipur, West Bengal, India, reviewing the cases of five patients.

In our cases we try to elaborate data regarding the age of occurrences, presentation, and behaviour of tumour and prognosis of MCC.

The patients' profiles, clinical presentation, age of occurrence & the tumour characteristics including histopathological and immunohistochemical profiles are similar to the cases presented in other parts of world.

Keywords: merkel cell carcinoma, neuroendocrine tumour, skin tumour.

GJMR-C Classification : NLMC Code: QZ 365, WP 460

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Merkel Cell Carcinoma : An Indian Experience

Dr. Biswanath Paul [α], Dr. Bhaskar Mitra [σ], Dr. Mallika Pal [ρ], Dr. Tarak Nath Saha [ω] & Dr. Ashok Maiti [¥]

Abstract- Merkel cell carcinoma [MCC] is a rare neoplasm of skin with an aggressive behaviour and unfavourable prognosis. MCC mainly occurs in the elderly, in the sun exposed areas of skin. Indian's experiences regarding patients' profiles, clinical presentation, age of occurrence, pattern of tumour are very limited as well as challenging.

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I. Introduction

Merkel cell carcinoma(MCC) is a rare primary cutaneous neoplasm with epithelial and neuroendocrine differentiation and it has been first described by Toker in 1972[1] as the "trabecular carcinoma of the skin". This neoplasm arises from the neuronal crest cells- Merkel cells which is situated in the basal layer of the epidermis. Though the exact cause remains unkown, sunlight has been reported to be a major risk factor. Common associations include in situ or invasive squamous cell carcinoma, basal cell carcinoma indicating its origin from multipotent stem cells of ectodermal derivation. High occurance of this tumour in organ transplant receipients and in immunocopromised patients indicates the role of immunosuppression in its etiogenesis(2). The reported annual incidence of MCC ranges from 0.2 to 0.45 per 100000[3]. It is mainly a disease of the Caucasian race. The annual age-adjusted incidence of MCC is 0.23 per 100,000 for whites and 0.01 for blacks[4]. Age of occurrence in elderly with mean age at presentation being around 75 years[5]. Only a few cases reported before the age of 50, and are usually related to immunosuppression[6]. Many cases have been reported worldwide, majority of which are Caucasians, while Indian experience regarding incidence of MCC, clinical presentation, age of occurrence and pattern of tumour are very limited. So in this article we tried to share our experience of such few rare occurrences in a tertiary care hospital of eastern India.

Author α σ ρ ω: Department of Pathology. Midnapore Medical College & Hospital, Paschim Medinipur. West-Bengal, India.
e-mail: bhaskarmitra12@gmail.com
Author ¥: Cancer Detection Centre Midnapore Medical College & Hospital, Paschim Medinipur West-Bengal, India.

II. Material and Methods

These retrospective observations were performed in a tertiary care hospital in Paschim Medinipur, West Bengal, India between 2009 -2011. We included patients with a pathologic diagnosis of MCC coming from neighbouring rural areas to this hospital. Patient characteristics, clinical features of the lesion (i.e. site, size, tenderness, colour and growing time), stage at presentation, and clinician's impression at the time of biopsy were reviewed. Tissue specimens were obtained from the Department of Surgery, Midnapore Medical College, Paschim Medinipur, West Bengal. Table 1 & 2 represents the patients & tumour characteristics respectively. The tissue samples were routinely embedded in paraffin and processed at the Department of Pathology, Midnapore Medical College Hospital, stained with Haematoxylin and Eosin stain and immunohistochemicaly studied with CK-20, chromogranin, neuron specific enolase (NSE) and TTF-1 as markers to confirm the diagnosis.

Table 1 : Patient's characteristics

		Number of cases (%)
Age	< 50 years	1(20%)
	60- 70 years	4 (80%)
Sex	Male	3(60%)
	Female	2 (40%)
Race	Asian	5
Occupation	Farmer	5
Immunocompromised	Yes due to CLL	1
	No	4

Table 2 : Tumour characteristics

		Number of cases (%)
Site of tumour	Head & neck	2(40%)
	Upper limb	2(40%)
	Lower limb	1(20%)
Size of tumour	< 2 cm	3 (60%)
	>2 cm	2(40%)
Shape of tumour	Nodular	4 (80%)
	Ulcerative	1 (20%)
Lymph node involvement	Yes	2(40%)
	No	3(60%)

III. Result

Patient characteristics, as detailed in table no 1, shows that during the study period 5 patients were came with a skin lesion, among them one was younger than 50 year, and others were older than 60 year. There was a slight male predominance with a ratio of 1.5:1 (60% male and 40% female). In this study all patients were Asian and farmer with history of occupational sun exposure. One patient had history of immunosuppresion due to pre-diagnosed CLL.

As mentioned in Table 2, most lesions appeared on sun-exposed skin. Most common site of the primary tumour was head & neck region (40%) and upper limbs (40%), followed by lower limbs (20%) (Fig-1). Size of the tumours were variable ranging from 1.5 cm to 4.6 cm. Primary tumour diameter of less than 2 cm was found in 3 cases and that of more than 2 cm in 2 cases. Four patients presented with a nodule covered by intact skin, while more advanced tumour form was found in one with ulcerating growth. The lesions were painless, firm, and non-tender. Regional lymph node metastasis found in two patients. Three patients of the group having no regional lymph node metastasis were clinically diagnosed as benign lesion. Other two patients with lymph node enlargement were clinically suspected as malignant.

All patients were subjected to excision followed by histopathological examination. The prepared H&E stained slides exhibited diffuse pattern of infiltration of dermis by small round cells with an intact epidermis (Fig 2a, 2b & 2c), cells having scanty eosinophilic cytoplasm and round vesicular nuclei with fine granular chromatin (Fig 2d). In immunohistochemical examination, the tumor cells showed typical perinuclear dot like positivity for cytokeratin 20 (Fig- 3a), negative for TTF-1 (Fig- 3b), as well as immunoreactive for chromogranin (Fig- 3c) and NSE (Fig- 3d). On the basis of these histological and immunohistochemical features, diagnosis of Merkel Cell Tumor were established. Then additional excisions were performed to achieve margins 3 cm wide and 1–2 cm deep along with lymph node dissection. The patients were evaluated with a CT scan for staging. CT scan reports revealed regional lymph node metastasis in two patients having primaries more than 2 cm in size; others were negative for metastasis. All patients were treated with post-operative radiotherapy. The follow-up were done through CT scan. Two patients died 1 year later during the follow-up period.

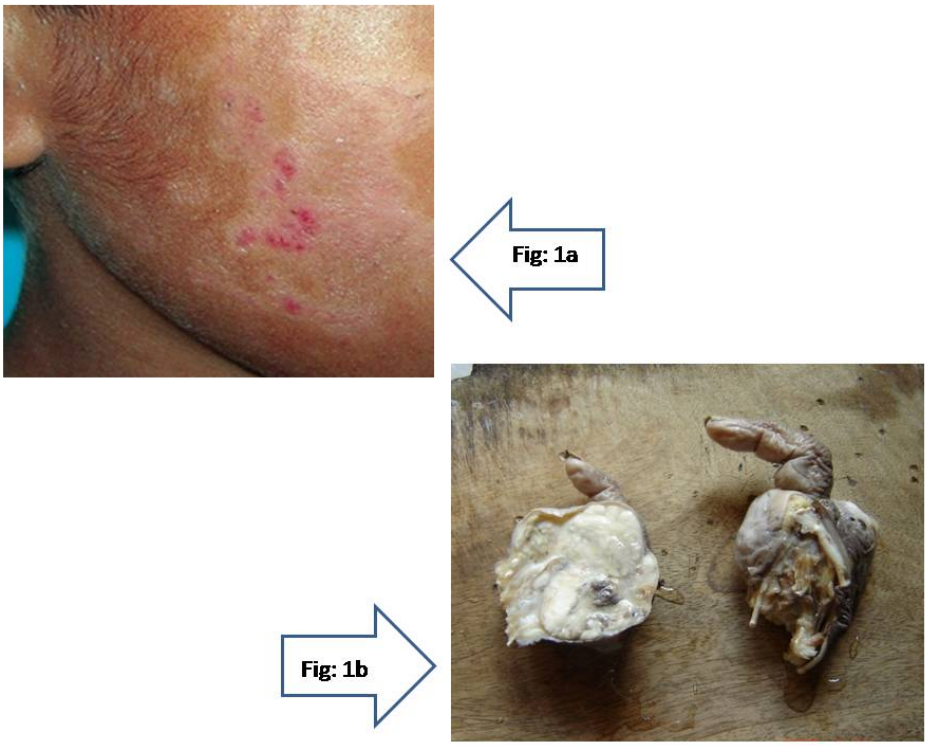

Figure 1

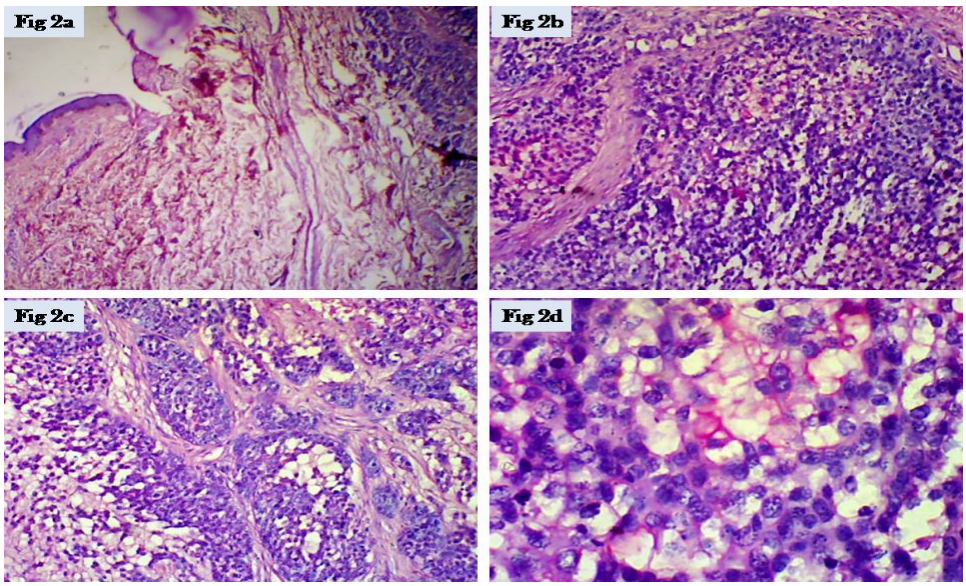

Figure 2

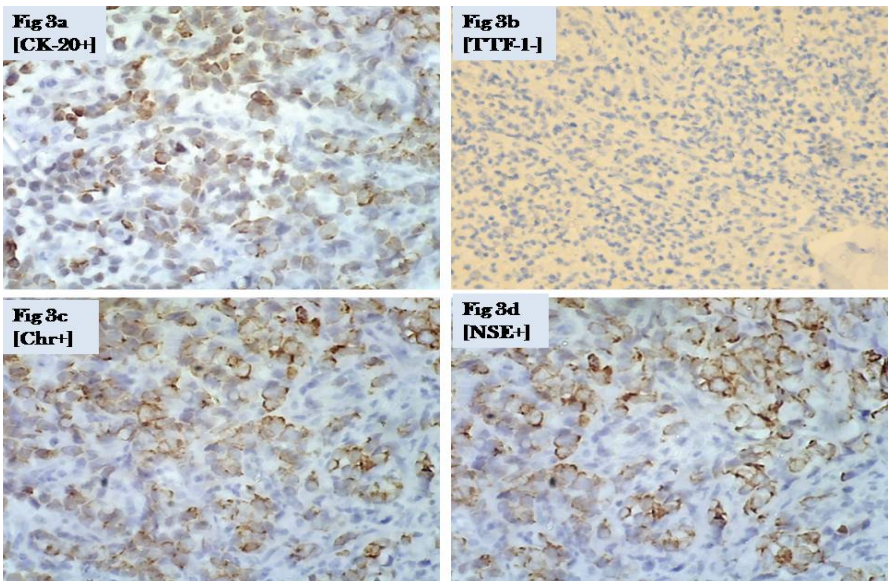

Figure 3

IV. Discussion

Merkel cell carcinoma is a rare neoplasm of skin with an aggressive behaviour and unfavourable prognosis[7]. Many reported cases found in literature are mainly in white[4]. Data regarding the occurrences, presentation, behaviour of tumour and prognosis is still limited in Indian subcontinent. In our study we found only five cases of MCC in three years of span (2009-2011). It is mainly a tumour of elderly[5], few cases found before age of 50 years were associated with immunosuppression[6]. In our study, among five cases four (80%) were within age range of 60-80 years and one (20%) younger than 50 years, who had previously diagnosed CLL. According to Brenner et al incidence of CLL as second neoplasm is 15.9% & majority of this second neoplasm preceded the diagnosis of MCC[8]. Here the occurrence of MCC was slightly higher in male with M:F ratio of 1.5:1, which also follows Heath et al study[9]. Constant sun light exposure is obvious in all patients as they all are farmer by occupation. Also they presented with tumours involving head & neck, upper limbs and lower limbs, supporting the possible contributory role of sun light in development of MCC, as shown by Miller & Rabkin in their study that incidence of MCC is increased with the exposure of solar UVB ray[9,10]. Van Gele et al also shows a UV-B–induced C to T mutation in MCC cell line11, suggesting that sun exposure plays a leading role in the pathogenesis of this tumour. However, this does not provide a satisfactory explanation, because MCC has been reported in non-sun exposed sites.

MCC usually presented as a non-tender nodular growth, sometimes as an ulcerated lesion[7]. Most of the tumours are approximately 2 to 4 cm in diameter[12]. Clinically correct diagnosis is made rarely, because

these lesions can resemble many other tumours. The clinical differential diagnosis often includes basal cell carcinoma, squamous cell carcinoma, pyogenic granuloma, keratoacanthoma, amelanotic melanoma, adnexal tumor, clear cell acanthoma, lymphoma, and metastatic carcinoma[13].

On gross examination it shows grey coloured cut surface[14] (Fig-1). Microscopically tumour occurs in the dermis, sometimes in subcutaneous tissue with an intact overlying epidermis[7], though epidermal involvement by the tumour is also reported previously[15]. Tumours are composed of small round cells arranged in diffuse and sometimes in trabecular pattern. The cells have scanty eosinophilic cytoplasm and round vesicular nuclei with fine granular chromatin & multiple nucleoli. Mitotic count is high[16,17]. Immunohistochemically MCC are positive for low molecular weight keratin (CK20), chromogranin & neuron specific enolase[7,18]. The CK20 shows typical perinuclear dot like positivity[7]. MCC are negative for TTF-1[19, 20]. In our present study all cases show similar above mentioned histological & immunohistochemical features.

Different types of chromosomal abnormalities have been reported in this tumour. Trisomy of chromosome 6 & 1 are most typical[21, 22, 23] along with partial & complete trisomy of chromosome 11[24, 25].

Due to rarity of the tumour there are multitudes of treatment protocols. The treatment depends on the stage of the tumour at the time of presentation. Patient with a localized tumour surgery with excision of 3 cm wide margins and 2 cm depth is considered as a gold standard method.[26, 27]. Postoperative radiotherapy in patients without lymph node metastasis is still controversial. But due to high rate of local relapse, 45-60 Gy[26, 28] is used routinely to the area of lesion to decrease the local recurrence[29]. The lymph node involvement is an important prognostic factor of this tumour. Its involvement decrease the survival rates from 88% to 50% and it is evident in 50% -70% of all patients within 2 years of diagnosis[30]. So sentinel node biopsy[31] and routine lymph node dissection[27, 32] is strongly recommended along with postoperative radiotheraphy in case of its metastasis[33]. Other poor prognostic factors are tumour size >2 cm, male sex, age>60 years & immunosuppression[26, 29, 32].

V. Conclusion

We reported five cases of merkel cell carcinoma, arising in different parts of the body, from surrounding areas of Paschim Medinipur district in eastern India. Patients' profiles and tumour characteristics including histopathological findings & immunohistochemical patterns are similar to those presented in other parts of world. A strong clinical suspicion, clinicoradiological correlation and histopathological and immunohistochemical confirmation are required for proper evaluation along with wide margin resection with or without postoperative radiotheraphy & lymph node dissection.

VI. Acknowledgement

The authors gratefully acknowledge the assistance of the laboratory staffs of the Department of Pathology, Midnapore Medical College and Hospital.

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Opportunistic Infections Vs Immune Suppression Among HIV Seropositive Individuals in East Godavari District, Andhra Pradesh

By Ankam SS Rajendra Babu, T.Jaya Chandra, Budida Ramachandramouli & Dr. R.Lakshmi Kumari
GSL Medical College, Rajahmundry, India

Abstract- *Aim:* To detect the opportunistic infections (OIs) and level of immune suppression in HIV sero positive patients in East Godavari district, Andhra Pradesh.

Material and methods: Study was conducted in the department of Microbiology, Rangaraya Medical College, Kakinada, referral center for HIV diagnosis and treatment by NACO. Study period 27 months, January 2011 to April 2013. Clinical specimen include stool, sputum, CSF, lymph node aspiration, swabs from oral cavity, blood were collected from 178 confirmed HIV seropositive individuals.

Results: Male female ratio was 1.41: 1. More number of HIV positive cases were seen in the age group of 31- 40 years (34%) and fever (72%) is the common clinical symptom. Mycobacterium tuberculosis (51%) was most commonly isolated pathogen, followed by Candida (39%), Cryptosporidium parvum (24%) with mean CD4 counts 231, 160 and 72 cells / µl respectively. Poly microbial infections were seen in 34% of the study volunteers.

Keywords: *HIV, opportunistic infections (OIs), mycobacterium, candida.*

GJMR-C Classification : *NLMC Code: WC 140*

Strictly as per the compliance and regulations of:

Opportunistic Infections Vs Immune Suppression Among HIV Seropositive Individuals in East Godavari District, Andhra Pradesh

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Conclusion: OIs are the major cause of mortality and morbidity in HIV positive patients. So early and correct diagnosis of OIs is required for proper disease management.

Keywords: HIV, opportunistic infections (OIs), mycobacterium, candida.

I. Introduction

The unique pathogenesis of HIV virus and drop in CD4 count are the two aspects responsible for the emergence of opportunistic infections (OIs) in HIV patients [1]. Due to decrease in immunity, people with HIV are prone for OIs and these infections are recognized as common complication [2, 3, 4]. OIs are major cause of morbidity and mortality in HIV patients [5, 6].

In the HIV positive individuals the incidence of OIs is reduced dramatically with introduction of Anti retroviral treatment (ART). But ART is not available to all in the resource limiting countries like India [7]. AIDS is the most important problem in 20th century [3] and leading cause of death. So care has to be taken to prevent and treat the OIs.

Various microorganisms cause OIs in HIV patients. As per the available literature tuberculosis, candidiasis, cryptosporidium diarrhoea, cryptococcal meningitis, pneumocystis carinii pneumonia are some of the common OIs in the HIV sero positive individuals[8]. The frequency of OIs may not be same in all countries and differ within the country. In resource limited, developing and high HIV burden countries like India the incidence and severity of OIs is high. But the literature which is available in this regard is limited especially with the correlation of immune suppression.

AIDS caused by HIV may not be curable. But the OIs can be treated. Hence identification of OIs causing pathogens is very important and essential in HIV patients for disease management. This not only prolongs the life of HIV individuals but also improve the quality of life.

We conducted a study to identify OIs causing microorganisms in HIV patients of East Godavari district in relation to the immune status.

II. Material and Methods

Study was conducted in the department of Microbiology, Rangaraya Medical College (RMC), Kakinada, for 27 months, study period January 2011 to April 2013. RMC, a National AIDS Control Organization (NACO) referral center for HIV diagnosis and treatment in East Godavari district, Andhra Pradesh. So HIV seropositive patients from different parts of district were included in the study.

Study population consists of HIV positive patients, both genders. Informed consent was taken from all the individuals in the presence of witness if required i.e. in case of minors and illiterates. Based on clinical condition and patient status various clinical samples were collected. This include stool, sputum, CSF, lymph node aspiration, swabs from oral cavity, blood.

Stool samples were collected in a wide mouth bottle. All the volunteers were instructed clearly that stool sample should not get contaminated with urine. Trophozoites, cysts, larva and helminthic ovum were identified in the stool samples by observing saline,

Author α: Department of Microbiology, Rajiv Gandhi Institute of Management and Sciences, Kakinada.
Author σ: Department of Microbiology, GSL Medical College, Rajahmundry: 533296.
Author ρ: Department of Clinical Immunology and Rheumatology, Hannover Medical School, Hannover, Germany.
e-mail: chanduthegreat@rediffmail.com
Author ω: Department of Microbiology, Siddardha Medical College, Vijayawada.

lugols iodine mount and LPCB mount[9, 10]. In addition stool smears were stained by modified ZN staining[10] to identify protozoan parasites like *Cryptosporidium parvum*, *Isospora belli*, Cyclospora. The predominant bacteria cause enteric infection, Salmonella, Shigella, *Vibrio choleare* were isolated and identified by inoculating stool sample on MacConkey agar, selective media i.e. XLD / DCA/ TCBS and growth is identified by various biochemical reactions as per the standard protocols[11, 12].

Sputum samples were collected as per Jaya Chandra et al[13] i.e three deep breaths followed by a deep cough. Minimum 5ml of sputum sample was collected. Immediately after collection, smear was prepared and stained by Ziehl Neelsen staining as per Revised National Tuberculosis Control Programme (RNTCP) guidelines[14]. After smear preparation, sputum samples were concentrated and decontaminated by NALC NaoH method[15] and the deposit was inoculated in 2 sets of LJ media and incubated at 37^0C. LJ culture reading was done as per RNTCP guidelines[16].

Oral swabs were inoculated on Sabourds Dextrose Agar (SDA) to identify candida which is the causative agent of oral candidiasis. For speciation, chlamydospore formation, germ tube test was done and the isolated candida was also inoculated on chrome agar[17].

CSF samples were collected from meningitis patients. Gram stained CSF smears were observed to identify the bacteria or fungal causative agents and India ink smears to identify cryptococcus capsule. CSF samples were inoculated on Chocolate agar, MacConkey agar, SDA and processed as per the standard protocol[10, 11].

In addition to clinical correlation, blood samples were tested for ELISA to identify Herpes simplex virus, Cytomegalo virus infections. CD4 counts were estimated by using BD FACS caliber machine as per the NACO guidelines.

III. Results

In the study male female ratio was 1.41: 1. More number of HIV positive cases were seen in the age group of 31- 40 years (Table: 1). Fever (72%) was the most common symptom followed by weight loss (69%) and chronic cough (44%) (Table: 2). *Mycobacterium tuberculosis* (51%) was the most commonly isolated pathogen, followed by Candida (39%), *Cryptosporidium parvum* (24%) (Table: 3) with mean CD4 counts 231, 160, 72 cells / μl. Poly microbial infections were seen in 34% of the volunteers (Table: 4), common in the patients with low CD4 counts. Figure shows the OIs in relation with immune stautus.

IV. Discussion

AIDS the only cause of HIV is the burning health issues of developing countries like India. India accounts for 1% of global burden. In AIDS patient's death is mainly due to OIs not by HIV. The important observation in our study is that OIs are seen in all the study subjects. So prevalence of OIs is 100%.

In our study majority of HIV positive cases were seen in the age group 31 – 40 years, 34% (60 out of 178 cases). Madhkar SS et al[4] and Patel SD et al[18] reported 53.3%, 52% HIV positivity in 31 – 40 years age group.

The present study showed that fever (72%) is the most commonly presenting symptom, followed by chronic cough (49%), chronic diarrhoea (48%), oral thrush (34%) and lymphadenopathy (25%). Most of the study subjects were presented with mixed symptoms. Findings of our study were very close with Patel SD[18], Gupta V et al[19], SK Sharma et al[20] showed fever is the most common complaint found in 64%, 51% and 70.4% followed by weight loss in 47%, 43% and 62.5% respectively. But weight loss (47.8%) is the common complication followed by PUO (36%) and chronic cough (33%), chronic diarrhoea (32.3%) as per Deorukhkar et al[3] report.

In the current study out of 178 HIV positive cases 98 patients expressed the promiscuous behaviour and all are heterosexuals with more than one sexual partner. Among these subjects HIV seropositivity was 84%. The available literature is also stated that heterosexual route is the commonest route of HIV transmission[4, 21, 22, 23].

This study revealed that Mycobacterium is the most common OIs causing agent in HIV patients, Mycobacterium isolation was 51%, followed by Candida (39%) and *Cryptosporidium parvum* (24%). Our findings were comparable with previous studies. In the available literature Mycobacterium was isolated in 50%, 47%, 56%, 47%, 57% and 59% respectively in Biswas Jyotir may et al[24], Vajapayee M et al[25], Singh A et al[26], Sanjeev Sinha et al[27], Nilanjan Chakraborthy et al[2] and Madkar SS et al[4]. In the current study both pulmonary tuberculosis (PT) and extra PT forms were seen (61, 11 cases) and mean CD4 count was 231 cells/ μl. As per Moore et al[28] one of the earlier studies, the mean CD4 count was 261 cells / μl in HIV positive patients with PT as OI and the reference range of CD4 counts were 250 to 500 cells / μl according to Crowe et al[29] (Table: 5).

Candida infection is second (39%) common next to Mycobacterium. This is confirmed with the studies of Patel SD[5] & Madhkar SS[4], reported candidiasis in 33% and 37.6% HIV positive patients respectively. *Cryptosporidium parvum* is the third (24%) common identified pathogen and very common diarrhoea causing agent. As per the Kulkarni et al[30] study 12% *Cryptosporidium parvum* were isolated in HIV positive patients.

The very important aspect of our study is correlation between CD4 counts and OIs. Increased incidence of OIs was seen in individuals with low CD4 counts. OIs rate was very high (58%) when the CD4

counts were < 200 cells / μl. As the CD4 count increases the incidence of OIs is decreased. The rate of OIs were 41% when CD4 counts were 200-500 cells / μl and it was just 1% when the CD4 counts were > 500 cells / μl. The reason is very clear, as the CD4 cell count is decreased, the individual may prone to other infections due to lowering of immune system [31]

V. Conclusion

Findings of our study showed that OIs are very common in HIV patients. As immunity decreases, OIs can increase. Tuberculosis is the common OI followed by candidiasis, cryptosporidium diarrhoea. Diagnosis of OIs may not only decrease the mortality in HIV patients, but also increase the quality of life. Hence the diagnosis of OIs should be given prime importance in HIV sero positive patients.

Table 1 : Age wise distribution of study population

Age	1-10	11-20	21-30	31-40	41-50	51-60	61-70
HIV cases (%)	3 (1.7)	3 (1.7)	57 (32)	60 (34)	36 (20)	13 (7.3)	6 (3.4)

Table 2 : Different clinical symptoms of study population

Symptom	%
Fever	72
Weight loss	69
Chronic cough	49
Chronic diarrhoea	48
Oral thrush	34
Lymphadenopathy	25

Table 3 : Various OIs causing microorganisms in the study population

S.No	OIs	Number	Mean CD4 count in cells / μl
1	**Bacteria**	**98**	**341**
	Mycobacterium tuberculosis	91 (51)	231
	Salmonella	2 (1.1)	293
	Pseudomonas aeruginosa	3 (1.7)	280
	Vibrio cholerae	2 (1.1)	560
2	**Fungi**	**73**	**143**
	Candida	69 (39)	160
	Cryptococcus neoformans	4 (2.5)	127
3	**Parasites**	**49**	**107**
	Cryptosporidium parvum	42 (24)	72
	Isospora belli	2 (1.1)	117
	Cyclospora	2 (1.1)	110
	Toxoplasma belli	3 (1.7)	130
4	**Viruses**	**17**	**93**
	Herpes simplex virus	9 (5)	110
	Cytomegalo virus	8 (4.5)	76

Table 4 : Mixed OIs in the study group (n= 178)

OIs	Number
Tuberculosis, Candida	14
Tuberculosis, Cryptococcus	08
Tuberculosis, cryptosporidium, Candida	07
Tuberculosis, Pneumocsytis pneumoniae	04
Candida, cryptosporidium	12
Candida, Tuberculosis, Salmonella	7
Candida, Pseudomonas	7
Cryptosporidium, HSV	1

Table 5 : Comparison of CD 4 counts

OIs	CD4 count in cells / μl		
	Present study	Moore et al [28]	Crowe et al [29]
Tuberculosis	231	261	250-500
Candidiasis	160	-	250-500
Cryptyosporidium diarrhoea	72	28	150-200
Crptococcal meningitis	127	63	75-125
Toxopalsma	130	44	75
HSV	110	195	75
CMV	76	37	50

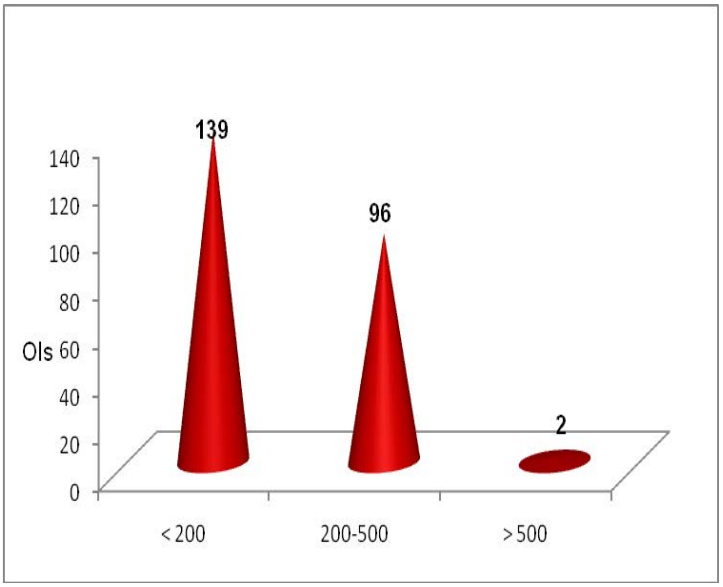

Figure showing OIs in relation with immune status

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Fatal Pulmonary Infection by a Multi-Resistant Strain of C.Laurentii in a Patient with Active Pulmonary Tuberculosis

By Aikaterini Marini, Alexios S. Antonopoulos, Claudia Lakoniti, Evangelia Kouskouni & Konstantinos Gerolymatos

Aretaieion University Hospital, Greece

Abstract- We report the first case of concomitant C. laurentii and M. tuberculosis pulmonary infection in a non-immunocompromised patient caused by a multiresistant C.laurentii strain and the fourth reported case of C.laurentii pulmonary infection up to now. We review the literature regarding C. laurentii pulmonary infections as well as its treatment.

Keywords: cryptococcus; tuberculosis; pulmonary infection; non-neoformans.

GJMR-C Classification : NLMC Code: WC 302, WF 300

Strictly as per the compliance and regulations of:

Fatal Pulmonary Infection by a Multi-Resistant Strain of C.Laurentii in a Patient with Active Pulmonary Tuberculosis

Aikaterini Marini [α], Alexios S. Antonopoulos [σ], Claudia Lakoniti [ρ], Evangelia Kouskouni [ω], & Konstantinos Gerolymatos [¥]

Abstract- We report the first case of concomitant *C. laurentii* and *M. tuberculosis* pulmonary infection in a non-immunocompromised patient caused by a multiresistant C.laurentii strain and the fourth reported case of *C.laurentii* pulmonary infection up to now. We review the literature regarding *C. laurentii* pulmonary infections as well as its treatment.

Keywords: cryptococcus; tuberculosis; pulmonary infection; non-neoformans.

I. Introduction

Our knowledge regarding non-neoformans cryptococcal infections has dramatically changed over the last years. While non-neoformans cryptococci had been previously considered as simple saprophytes, we now know that such cryptococci can be occasional pathogens, responsible for serious infections. *C.laurentii* together with *C.albidus* account for more than 80% of non-neoformans cryptococcal infections [1]. To our knowledge this is the fourth case of human *C.laurentii* lung infection and the first report of tuberculosis and *C.laurentii* co-infection of in a non-immunocompromised patient.

II. Case Report

An ex-tanner, 83-year-old man presented to our emergency department with a 2-week history of weakness, chills, dyspnea, productive cough, haemoptysis and fever up to 39.5°C. He had a history of arterial hypertension, COPD, chronic atrial fibrillation and had undergone a surgery for an in-situ large intestine tumor removal 6 years ago. Physical examination revealed respiratory distress (35 breaths / min), with ample crackles and wheezes on auscultation of both lungs, while chest x-ray revealed signs of left-upper lobe pulmonary infiltration. The patient was hemodynamically stable, however his arterial blood gases indicated mild hypoxemia (pO2=62mmHg, pCO2=42mmHg, SaO2=89%). The initial laboratory tests revealed an acute inflammatory status with leukocytosis (WBC=15390/μL, Neutrophiles=13670/μL), a three-digit erythrocytes sedimentation rate (ESR=119mm/h) and highly elevated C-reactive protein (CRP=292 mg/L). There were no laboratory findings of renal / liver impairment or electrolytes' abnormalities. Blood, urine and sputum cultures were obtained and a tuberculin test was performed. He was immediately treated with ceftriaxone (2g/day), bronchodilators and nasal oxygen as a possible lung infection.

The combination of positive tuberculin test (15mm) and the radiologic and clinical features indicated a high risk of tuberculosis infection (figure 1A). Chest computed tomography reinforced our initial suspicion of active pulmonary tuberculosis by revealing a cavitated opaque lesion of the left upper lobe, as well as bronchopulmonary infiltrations of the lower lobes in both lungs and the reed (figure 1B). On this basis anti-tuberculosis treatment was initiated (rifampin 600mg/day, isoniazid 300/day, ethambutol 2g/day and pyrazinamide 2g/day). The patient remained nonfebrile for the next 9 days and the clinical and laboratory findings were ameliorated (WBC=7340/μL, Neutrophiles=6010/μL, CRP=94.5mg/L, ESR=95 mm/h). Meanwhile two samples of blood and sputum cultures were obtained.

However, during the following days the patient's clinical state deteriorated, manifesting marked respiratory distress, tachypnea, fever accompanied with chills (up to 39.0°C) and lethargic mental status. Moreover there was a simultaneous alteration in laboratory findings (CRP=170mg/L, ESR=102mm/h). Blood and urine cultures were negative, while sputum cultures' analysis revealed *M. tuberculosis* suggesting active pulmonary tuberculosis. Furthermore, sensitive strains of *Klebsiella pneumonia* and *Citrobacter freundii* were also isolated in sputums as well as a rare strain of Cryptococcus, identified as *C. laurentii*. These findings suggested co-infection of the underlying pulmonary tuberculosis with a very rare and highly resistant type of non-neoformans Cryptococcus. For the newly isolated bacterial strains, ciprofloxacin (400mg x 2) was added based on the antibiogram's results. Importantly, the

Author α σ ρ ¥: Internal Medicine Department, IKA Hospital, Melissia, Greece. e-mail: antonopoulosal@yahoo.gr
Author ω: Department of Microbiology, Aretaieion University Hospital, Athens, Greece.

identified *C.laurentii* strain was multi-drug resistant to all known antifungal agents (amphotericin B: MIC>16000 μg/mL, fluconazole: MIC>129000 μg/mL, itraconazole: MIC>4000 μg/mL, voriconazole: MIC>8000 μg/mL). Given the above results, empiric antifungal treatment with liposomial amphotericin B (5mg/kg) and caspofungin (70 mg on the first day and 50mg/day subsequently) was initiated in addition to the anti-TB drugs. The aforementioned multidrug resistance of the isolated Cryptococcus strain was also confirmed by the second sputum culture analysis. A microscope image of the *C.laurentii* strain was also taken and is depicted in figure 2.

Despite treatment, the patient remained lethargic and febrile with deteriorating vital signs. A second CT scan depicted the presence of liquid in pre-existing pulmonary cavity and "ground glass" sign in the right upper lobe (figure 1C). No abnormal findings were observed in the brain and abdomen CT scan. Ultimately the active pulmonary disease led to respiratory failure and death after 23 days of hospitalization.

III. Discussion

Cryptococcus yeast is responsible for a series of very rare and life-threatening fungal infections in immunocompromised patients [2,3]. Cryptococcal infections are usually attributed to neoformans species, distributed in the air, soil, animal and plant organic residues [4]. *C. laurentii* along with *C. uniguttulatus*, *C. albidus*, *C. curvatus* and *C. humicolus* belong to non-neoformans cryptococci. *C. laurentii* and *C. albidus* are responsible for 80% of the non-neoformans infections [1]. Neoformans and non-neoformans species differ in capsule formation, melanin growth and antifungal resistance but their distinct classification remains a matter of debate.

Non-neoformans cryptococci were thought to be saprophytic and nonpathogenic to humans but incidence rates have importantly increased nowadays [1, 5, 6]. Interestingly, there are only 20 cases of *C. laurentii* human infections and only 3 affecting the lungs in immunosuppressed subjects [3, 6-9]. The yeast has been detected in normal skin, air, water, wood, soil, pigeon excrements, cheese, fruits, pork products, bean, wine andmilk of suffering from mastitis cows [10]. There have been no previous reports of *C. laurentii* lung infection in a non-immunocompromised subject neither of a co-infection with *M. tuberculosis*. Thus, the present case is of high clinical interest since we report a unique so far concomitant lung infection of *C. laurentii* and *M. tuberculosis* in a healthy subject.

Predisposing factors to *C. laurentii* infection are the presence of invasive devices (e.g. intravenous catheters, parenteral nutrition), the use of broad spectrum antibiotics, impaired cell-mediated immunity, leukemia, cancer, diabetes mellitus, HIV, prematurity, neutropenia, lymphopenia, immunosuppressive drug use and organ transplantation [1]. Extremely rare cases of "idiopathic CD4 deficiency" and congenital immunodeficiency have been also regarded as responsible for such infections. Our patient had no known defense impairment but he was diabetic and during his hospitalization he was treated with broad spectrum antibiotics. Furthermore, he carried central intravenous catheters for parenteral nutrition purposes.

Infection usually is acquired via the respiratory routes, alimentary tract and injured skin. C. laurentii may cause pneumonia, meningitis (2-9%), peritonitis, cutaneous infection, eye infection, invasive disease or fungemia. Fungemia of *C.laurentii* occurs mainly in cancer patients, neonates or as a complication of immunosuppressive therapy [1,11].

Pulmonary infection of *C. laurentii* can present as pneumonia, lung abscess or empyema [1, 8]. Typical radiographic findings include opaque or cavitated lesions, hilar enlargement, pleural fluid or an ARDS like pattern [1]. In our case, the chest radiography and a chest CT scan revealed a cavitated lesion with liquid levels and a typical pattern of pneumonia. Furthermore, the yeast has been previously isolated from sputum, pleural or abscess fluid and bronchial swab material [1]. Thus, its identification in sputum is regarded as a reliable one.

Pulmonary or oropharynx *C. laurentii* infection is rare and has been recorded only in immunity defense-impaired patients [3]. Only a previous *C. laurentii* lung infection has been reported in a subject with unknown underlying disease and two cases of *C. laurentii* presence in cadavers belonging to non-immunosuppressed subjects, where there was also a co-infection with *C. neoformans* strains. Our patient presented pulmonary *C. laurentii* infection which is the first reported in a living non-immunocompromised subject.

Additionally, this is the first case of simultaneous pulmonary infection of *C. laurentii* and *M. tuberculosis* in otherwise healthy or non-immunosuppressed subjects. Concomitant cerebral tuberculosis and cryptoccocosis are extremely rare in the literature, affecting only HIV patients [3].

There is no standard treatment for C. laurentii infection. In a number of series, *C. laurentii* has been successfully treated with amphotericin B (94%) or fluconazole [8]. In vitro evidence about the susceptibility of *C. laurentii* to antifungal agents, suggests that *C. laurentii* strains are mainly sensitive to amphotericin B and itraconazole. Itraconazole, ketoconazole and voriconazole are scarcely preferred, though itraconazole has better tissue bioavailability in lungs compared to fluconazole. Fluconazole is strongly indicated for fungemia due to *C. laurentii*. Drug susceptibility testing should be conducted before any commence of

treatment. Antifungal resistance is associated with melanin deposition of the strains [12] and is referred mainly for fluconazole and flucytosine. It has been also related to prior azole administration and other host comorbidities [1]. Nevertheless, in a previous study testing drug susceptibility of yeasts found in synanthropic bird faecal samples, *C. laurentii* was found highly resistant in 11 antimycotic agents [10]. *C. laurentii* found in our patient was also highly resistant to all common antifungals (fluconazole, amphotericin B, itraconazole, voriconazole). Importantly, this evidence highlights that these rare infections can be severe and even fatal, due to our inability to efficiently combat with these highly resistant *C.laurentii* strains. Apart from resistance to antifungal agents, advanced age and CNS involvement have been identified as poor prognostic factors [1].

The current report describes the first case of *C. laurentii* concomitant lung infection with *M. tuberculosis* in a non-immucompromised patient. *C. laurentii* has recently been recognized as an opportunistic fungal pathogen in immunosuppressed subjects but remains extremely rare in healthy subjects. Early suspicion and diagnosis are critical to prompt treatment. It is critical to remain vigilant for Cryptococcosis lung infections even in non-immunosuppressed patients as they may lethal and mimic other lung diseases.

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Figures and Figures Legends

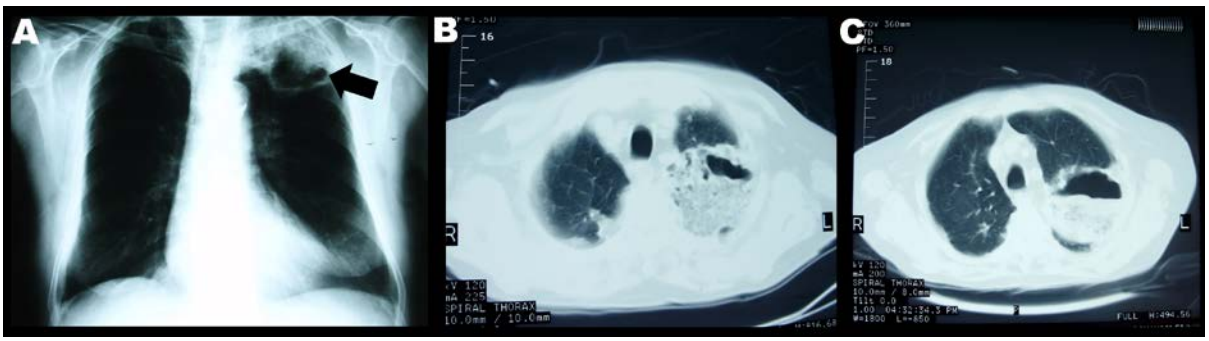

Figure 1 A : Posteroanterior chest radiograph shows left upper lobe consolidation and a cavitary opacity in the left upper lobe, **B.** Chest CT revealing caseous necrosis and ground glass opacities in the left upper lobe, **C.** Chest CT revealing a cavity with irregular borders and air-fluid levels, secondary to caseous necrosis, surrounded by thick outer wall

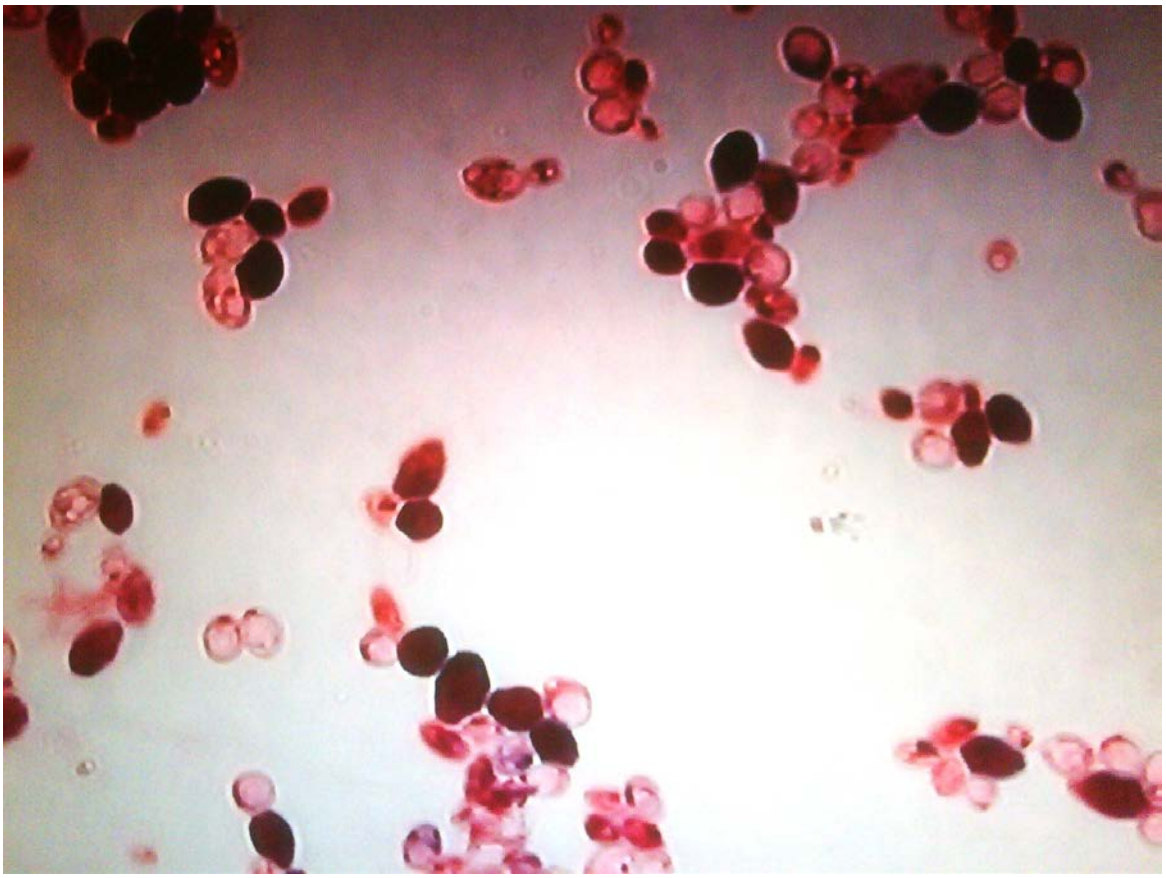

Figure 2 : The Gram's stain revealed spherical and elongated budding yeast-like cells without any pseudohyphae, identified as *C .laurentii*. India ink was also used for early visualization of the capsule that gave the characteristic "halo" around the cell (not shown here)

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Hyper-Immunoglobulin E Syndrome in a Neonate: A Case Report

By İlyas Yolbas, Velat Sen, Bilal Sula, Lokman Timuragaoglu & Hasan Balik
Dicle University, Medical School, Diyarbakir, Turkey

Abstract- Hyper-immunoglobulin E syndrome (Job syndrome) is a rare primary immunodeficiency with variable presentation, characterized by recurrent infections, facial dimorphism, eczema, scoliosis, joint hyper-extensibility, pathologic fractures, very high IgE (>2000 IU/mL), severe eosinophilia and variable impaired T cell function. We present a case of Hyperimmunoglobulin E syndrome in neonate with review of the literature. J Microbiol Infect Dis 2013; 3(3): 144-146.

Keywords: hyper-immunoglobulin E syndrome, recurrent infections, neonate.

GJMR-C Classification : NLMC Code: WF 250

Hyper-Immunoglobulin E Syndrome in a Neonate: A Case Report

Bir yenidoğanda Hiperimmunglobulin E sendromu: Olgu sunumu

İlyas Yolbaş [α], Velat Şen [σ], Bilal Sula [ρ], Lokman Timurağaoğlu [ω] & Hasan Balık [¥]

Abstract- Hyper-immunoglobulin E syndrome (Job syndrome) is a rare primary immunodeficiency with variable presentation, characterized by recurrent infections, facial dimorphism, eczema, scoliosis, joint hyper-extensibility, pathologic fractures, very high IgE (>2000 IU/mL), severe eosinophilia and variable impaired T cell function. We present a case of Hyperimmunoglobulin E syndrome in neonate with review of the literature. J Microbiol Infect Dis 2013; 3(3): 144-146.

Keywords: hyper-immunoglobulin E syndrome, recurrent infections, neonate.

Özet- Hiper-immunglobulin E sendromu (Job Sendromu), genellikle çok yüksek IgE (> 2000 IU/ml) seviyesi, şiddetli eozinofili, soğuk stafilokok cilt absesi ve pnömoni gibi tekrarlayan enfeksiyonlar, egzama, skolyoz, eklem hiperekstansibilitesi, patolojik kırıklar, tipik bir yüz görünümü, kraniyosinostoz ve değişken bozulmuş T hücre fonksiyonu ile karakterize nadir görülen primer □ubcla yetmezlik durumudur. Hiper-immunglobulin E sendromu yenidoğan ve diğer yaş grubunda farklı laboratuar bulguları, klinik belirti ve bulguları gösterebilir. Bu çalışmada soğuk Stafilokokal cilt abseleri, hafif yüksek total serum IgE düzeyi (146 IU/ml, normal: 0-8 IU/ml), yüksek □ubclass□l eozinofili (% 15) ve normal serum IgA, IgG, IgG □ubclass, IgM, C3 ve C4 seviyelerine sahip hiper-immunglobulin E sendromu olan onbeş günlük erkek hasta sunuldu.

Anahtar Kelimeler: hiper-immunglobulin E sendromu, job sendromu, yenidoğan.

I. Introduction

Hyper-immunoglobulin (Ig) E syndrome (HIES or Job Syndrome) is a rare primary immunodeficiency generally characterized by recurrent infections such as staphylococcal cold skin abscesses and pneumonia, eczema, scoliosis, joint hyperextensibility, pathologic fractures, a typical facial appearance, craniosynostosis, very high IgE, severe eosinophilia, and variable impaired T cell functions. The mechanisms responsible for hyperproduction of IgE and eosinophils in patients with HIES are presently unknown. Generally the onset of HIES occurs in children and elderly individuals.[1,2] HIES may have variable presentation, and laboratory values in different age groups.[3,4]

We present a 15-days old newborn with HIES whose only have staphylococcal cold skin abscesses eosinophilia and high immunoglobulin E levels.

II. Case Report

A fifteen-days-old male neonate born at 40 weeks of gestation by normal spontaneous vaginal birth to a 24 years-old mother without history of significant disease such as eczema or HIES in the family. The antenatal ultrasonography was normal. The patient was admitted to Dicle University Hospital at fifteenth day of his life, because of cold abscess that appeared 5 days before admission. On physical examination there was a 2x3 cm swelling compatible with cold abscesses in the anterior right knee area, right supraclavicular area, lateral right chest area and anterior left ankle area. He also had a characteristic facial appearance such a broad nasal bridge, cheilitis, thickened skin, and deep-set eyes with a prominent chin and forehead (Figure 1-3). There were no eczematous rash, scoliosis, fractures history, joint hyperextensibility and craniosynostosis on his physical examination and history. Yolbaş İ, et al. Hyper-immunglobulin E syndrome 145 J Microbiol Infect Dis www.jmidonline.org Vol 3, No 3, September 2013 The patient had slightly higher total serum IgE level (146 IU/mL, normal range: 0-8 IU/mL), high peripheral eosinophilia (15%) and normal serum IgA, IgG, IgG subclasses, IgM, C3 and C4 levels. The patient's other biochemical parameters were normal. The neonate underwent incision with pus aspirated which later grew Staphylococcus aureus. The skin biopsy showed eosinophil infiltration. Staphylococcal cold skin abscesses were treated with Ampicillin-sulbactam after drainage. HIES was diagnosed by clinically and laboratory tests, because there are no genetic or other confirmatory tests available in Turkey. The patient's computed tomography of the lungs was normal. The patient's Dual-energy X-ray absorptiometry test was found normal. The patient was discharged after two week from the hospital without any complications.

Author α σ ω: Department of Pediatrics, Dicle University, Medical School, Diyarbakir, Turkey. e-mail: ilyasyolbas@hotmail.com
Author ρ: Department of Dermatology, Dicle University, Medical School, Diyarbakir, Turkey.
Author ¥: Diyarbakir Children's Hospital, Diyarbakir, Turkey.

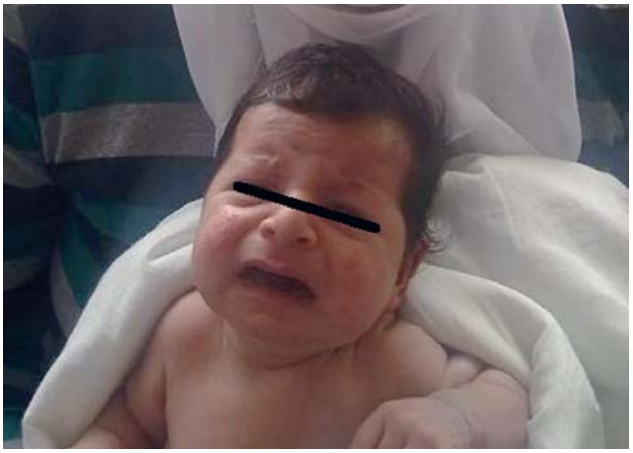

Figure 1 : Characteristic facial appearance such a broad nasal bridge, cheilitis, thickened skin, and deep-set eyes with a prominent chin and forehead, and Staphylococcal cold skin abscesses on right supraclavicular area

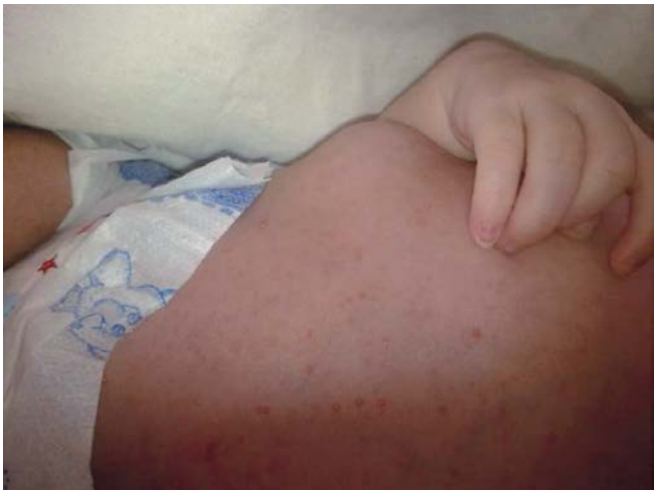

Figure 2 : Staphylococcal cold skin abscesses on lateral right chest area

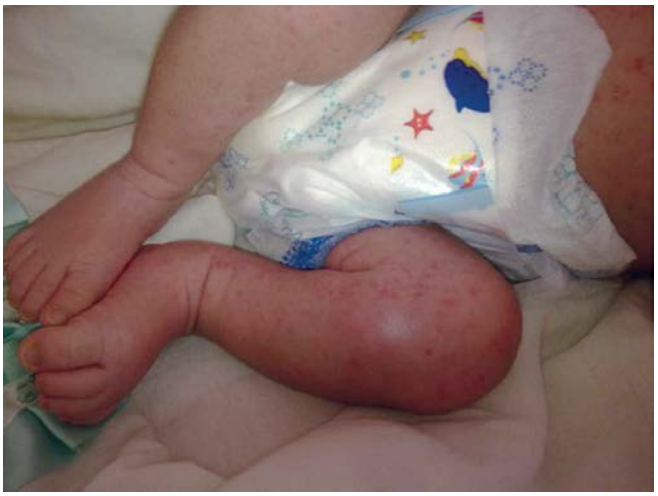

Figure 3 : Staphylococcal cold skin abscesses on anterior

III. Discussion

HIES is a multi-system disorder with a wide range of clinical phenotypes and signs, including skeletal, connective tissue, and vascular abnormalities.[3] Most of patients with HIES suffer from recurrent staphylococcal infections of skin and lungs.[4] Generally recurrent pyogenic pneumonias start in early childhood, and the most common infecting organisms are Staphylococcal aureus, Haemophilus influenzae and Streptococcus pneumoniae, Also mucocutaneous candidiasis is common in HIES.[4] Musculoskeletal abnormalities of HIES are scoliosis, osteopenia, minimal trauma fractures, hyperextensibility and degenerative joint disease.[3,5] The patients with HIES may have problem with development of their teeth.[6] Our case had multiple cold skin abscesses in the various regions of body but had no other stigmata of HIES at this age. Characteristic facial appearance of HIES include broad nasal bridge, cheilitis, thickened skin, and deep-set eyes with a prominent chin and forehead3. Our case had the similar characteristic facial appearance such as broad nasal bridge, cheilitis, thickened skin, and deep-set eyes with a prominent chin and forehead.

The two most consistent laboratory abnormalities in HIES are eosinophilia and elevated serum IgE. Over time, the serum IgE may decline in adults or may increase in newborn.[3] Demirci at al[7] found that IgE level of a two-month-old patient with HIES was 75.3 IU/ml (Range: 15-32 IU/ml), But in the same patients' they found IgE level 13,000 IU/ml after eight months. The patients with HIES have normal serum IgM, IgG, and IgA levels.[3] Our case have had slightly higher total serum IgE level as 146 IU/ 146 Yolbaş İ, et al. Hyper-immunglobulin E syndrome J Microbiol Infect Dis www.jmidonline.org Vol 3, No 3, September 2013 mL (Normal range: 0-8 IU/mL) and high peripheral eosinophilia (15%).

The diagnosis of HIES is usually made based on characteristic facial appearance and clinical features associated with high serum IgE level and eosinophilia.[5] Our patient had some of the characteristic features and laboratory findings. However definitive diagnosis is made on genetic basis such as STAT3.

Management of HIES currently revolve around prevention and treatment of infections. There is no cure for HIES at present. Therapy includes drainage of cutaneous abscesses followed by intravenous antibiotic therapy directed against mostly staphylococcal aureus. Prophylactic antibiotics and specific treatment is based on organ involvement. Immunoglobulin replacement therapy and some other treatments such as IFN-g, IFN-a, histamine-2 antagonists, and cyclosporine have been tried, which seem to be useful in the management of patients with HIES.[2,8] Prophylactic antibiotic or antifungal prophylaxis (e.g., trimethoprim-sulfamethoxazole or fluconazole) should be recommended in the patients

with HIES with recurrent sinopulmonary, cutaneous infections, mucocutaneous candidiasis and invasive fungal infections.[8]

In conclusion, HIES may present with some features in the newborn baby. Recognition of leading signs of the disease will provide early diagnosis and prophylactic measures.

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Staphylococcus aureus and its Antimicrobial Susceptibility Pattern in Patients, Nasalcarage of Health Personnel, and objects at Dessie referral hospital, Northern Ethiopia

By Zerfie Taddesse, Moges Tiruneh & Mucheye Gizachew
University of Gondar, Ethiopia

Abstract- *Introduction:* Staphylococcus aureus is one of the most common causes of healthcare and communityassociated infections. Its remarkable ability to acquire antimicrobial resistance mechanisms and advantageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings.

Objective: To determine prevalence of Staphylococcus aureus and antibacterial susceptibility patterns in patients, nasal carriage of health personnel and objects of Dessie Referral Hospital.

Methods: Cross sectional study was conducted at Dessie Referral Hospital from February 01 to May 30, 2013. Using a convenient sampling technique, 180 specimens of pus, blood, nasal swab and swab from hospital objects were collected and cultured by standard procedure. Growth identification was based on colony morphology, Gram staining and results of biochemical tests. Antibacterial susceptibility testing was done by disk diffusion method on Mueller-Hinton agar.

Keywords: *Staphylococcus aureus, antimicrobial susceptibility, ethiopia.*

GJMR-C Classification : *NLMC Code: QW 161.5.S8*

Staphylococcus aureus and its Antimicrobial Susceptibility Pattern in Patients, Nasalcarage of Health Personnel, and objects at Dessie referral hospital, Northern Ethiopia

Zerfie Taddesse [α], Moges Tiruneh [σ] & Mucheye Gizachew [ρ]

Abstract- ***Introduction:*** *Staphylococcus aureus* is one of the most common causes of healthcare and community-associated infections. Its remarkable ability to acquire antimicrobial resistance mechanisms and advantageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings.

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Result: Overall prevalence of *Staphylococcus aureus* was 40.5% and its occurrence in inpatients, health personnel and objects was 57.5%, 40% and 34.3% respectively. Penicillin G (90.4%), nalidixic acid (93.2%), and amoxicillin (82.9%) showed high level of resistance, whereas, gentamicin (84.3%)), tetracycline (62.9%) chloramphenicol (63.6%), ciprofloxacin (61.6%), and kanamycin (64.4%) were relatively effective against *Staphylococcus aureus* infection. Vancomycin exhibited 100% susceptible in all study subjects.

Conclusion: *Staphylococcus aureus* is still the most common cause of nosocomial infection and multi-resistant was very high and most of the isolates showed high levels of resistance to commonly used antimicrobials. In the absence of diagnostic bacteriologic services, vancomycin and gentamicin are the best therapeutic options to treat *S. aureus* infections.

Keywords: *Staphylococcus aureus*, antimicrobial susceptibility, ethiopia.

I. Background

Staphylococcus aureus (S. aureus) belongs to the genus Staphylococcus, which has more than 20 species. *S. aureus* is a Gram-positive coccus, catalase and coagulase positive and causes diseases through the production of toxins and enzymes and through direct invasion and destruction of tissues (1). It is one of the most common causes of healthcare- and community-acquired infections, such as localized cutaneous and life threatening systemic infections. Although most community infections are treated in the outpatient setting, some invasive infections, including bacteremia, septic arthritis, toxic shock syndrome, osteomyelitis, and endocarditis, have devastating complications and may require hospitalization (2, 3). Hospitalized patients are unusually at high risk of infection for various reasons, and tend to be more susceptible to infections. *S. aureus* causes more sever diseases in immunocompromised patients than in immune competent ones (4).

S. aureus is one of the most successful and adaptable human pathogens. Its remarkable ability to acquire antibiotic-resistance mechanisms and advent-ageous pathogenic determinants has contributed to emergence of infections in both nosocomial and community settings. However, because of different selective pressures, several notable differences exist between nosocomial-and community-acquired strains (5). Healthcare workers are potential source of hospital-acquired infections. Pathogens are transmitted by carriage on hands from inanimate objects present in the hospital setting (6). However, the role of fomites and the inanimate hospital environment (e.g. surfaces and medical equipment) in the transmission of healthcare associated infections is controversial (7). Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections. It is a major risk for the development of infection in patients undergoing hemodialysis, continuous ambulatory peritoneal dialysis, surgical patients, and patients with intravascular devices (8).

Multidrug-resistant strains of *S. aureus*, particularly methicillin resistant *S. aureus* (MRSA), pose a major clinical and epidemiological problem in hospitals, as they are easily transferred among hospital staff and patients(9). Antimicrobial resistance among nosocomial pathogens is a significant problem in many countries with severe consequences including increased medical

Author α: Department of Bacteriology, Dessie Regional Research Laboratory, Dessie, Northeast Ethiopia.
Author σ ρ: Department of Medical Microbiology, School of Biomedical and Laboratory Sciences, University of Gondar.
e-mail: muchegiza@gmail.com

costs, morbidity and mortality of patients (10). Since the first isolation of MRSA in the United Kingdom in 1961 (11), increasing rates of methicillin resistance among *S. aureus* strains have been a cause for concern, especially in developed countries. Until recently, vancomycin was believed to have retained activity against all strains of MRSA; therefore, the spread of MRSA has led to increased vancomycin usage and hence increased selective pressure for the development of resistance (12). The first report of MRSA in Ethiopia was made from 1987-1988 and the overall MRSA isolation rate was 31% while 71% out of the MRSA strains were multiple drug resistant (13). Nosocomial infection causes substantial morbidity and mortality, prolong hospital stay of patients, and increase direct patient-care costs. Widespread uses of antibiotics, together with length of time over which they have been available have led to major problems of resistant organisms. *S. aureus* as a cause of various nosocomial infections has not been recognized in Dessie Referral Hospital. Studying staphylococcal nosocomial infections in the area is essential for early prevention and control, correct diagnosis and treatment, and reducing morbidity and mortality of hospitalized patients owing to this infection. The aim of this study was therefore to assess prevalence of *S. aureus* and its susceptibility pattern to antimicrobials in inpatients isolates, nasal carriage of hospital personnel and hospital objects of Dessie Referral Hospital.

II. Material and Method

a) Study area

The study was conducted in Dessie, capital of South Wollo Zone in Amhara Regional State Northern Ethiopia, located 401 km far from Addis Ababa, on the way to Asmara. This town has a latitude and longitude of 11°8N 39°38E/11.133°N 39.633°E with an elevation of between 2,470 and 2,550 meter above sea level. The town has an estimated total population of 151,094 of whom, 78,203 are women (**14**). Dessie has one referral hospital, three general hospitals (private), three health centers, five higher clinics (private) and one regional health research laboratory where culture and susceptibility tests are performed.

b) Study Design and period

A cross sectional study was conducted from February 01 to May 30, 2013.

III. Population

a) Source population

All patients visited Dessie referral hospital, all health personnel who were working in this hospital and Objects (blankets, floor and cupboards) which were being used by patients in the hospital.

b) Study population

All patients who were admitted to Dessie referral hospital and who had developed signs and symptoms of hospital acquired infection after 48hs of admission during the study period, all health personnel who were working in inpatient wards of the hospital and who were willing to participate in the study and the objects (blankets, cupboards and floor) which were being used by patients in the hospital.

c) Inclusion criteria

Patients who had signs and symptoms of hospital acquired infection after 48 hours of admission to hospital, and health personnel who had not antimicrobials within seven days of specimen collection during the study period.

IV. Variables

a) Dependent variable

Prevalence and antimicrobial susceptibility pattern of *S. aureus*

b) Independent variables

Sex, age, hospitalization, catheterization, surgery

c) Sample size determination and sampling technique

Convenient sampling technique was used. All the 40 patients who had developed signs and symptoms of hospital acquire infection during the study period were included in the study. Thirty five volunteer health personnel in five inpatient wards (medical, surgical, gynecology, pediatric and orthopedic) were also included. In addition, 105 specimens were taken from Objects (blanket, cupboards and floor) that could be touched with hands of health personnel and patients within the five wards.

V. Data Collection and Laboratory Methods

a) Socio-demographic data collection

Data on socio-demographic characteristics from each study participant was collected using pre-tested questionnaire guided interview.

b) Specimen collection

Specimens were collected from the study participants using the standard operational procedures. Thirty six swabs of wound secretions were aseptically obtained from patients after patients were diagnosed as wound sepsis by a physician. The specimens were collected with sterile cotton swabs before the wound was cleaned with an antiseptic solution and 10ml of four blood samples were aseptically collected from each patient, and mixed into blood culture bottle containing 90ml of nutrient broth. Nasal swabs were taken from 35 health personnel with sterile cotton swab. A separate sterile cotton swab was passed into the anterior nares of both the nostrils and rotated in both directions and then

placed into sterile test tube. One hundred five specimens were collected from Objects (blanket, cupboards and floor). Sterile cotton swabs moistened with normal saline was rotated against the surface of objects to obtain specimens. All collected specimens were labeled and transported to Dessie Regional Health Research Laboratory for culturing and antimicrobial susceptibility testing.

c) Bacterial isolation and identification

Swab specimens were cultured onto mannitol salt agar and incubated at 35-37°c for 24 hrs. Each culture was read and then sub-cultured onto blood agar and incubated at 35-37°c for 24 hrs. Blood samples were incubated at 35-37°c for 7-14 days (until growth was seen) and growth was sub-cultured on mannitol salt agar. Identification of growth was based on colony morphology, Gram staining and appropriate biochemical test. *S. aureus* is a gram positive, beta hemolytic, catalase, and coagulase positive.

d) Antimicrobial susceptibility testing

Antimicrobial susceptibility testing of isolates was performed using disk diffusion method on Muller-Hinton agar plates as per the National Committee for Clinical Laboratory standards (15). Single colony was selected and emulsified in 3ml sterile normal saline solution in a sterile test tube. The turbidity of the suspension was then adjusted to the density of a barium chloride standard (0.5 McFarland) in order to standardize the size of inoculums. A sterile cotton swab was dipped into the standardized suspension of the bacterial culture, squeezed against the sides of the test tube to remove the excess fluid and inoculated onto Mueller-Hinton agar and allowed to dry the flood. Thereafter, antimicrobial discs were placed on the agar with forceps and gently pressed down to ensure contact. The plates were then allowed to stand for 30 minutes for diffusion of active substance of the agents. Plates were inverted and incubated at 35-37°c for 24 hrs. An inhibition zone diameter of each antimicrobial was then measured and interpreted as 'Resistant', 'Intermediate' and 'Sensitive' by comparing with recorded diameters of a control organism, ATCC25923 (16). Antimicrobials used, include oxacillin (1μg), vancomycin (30 μg), penicillin G (10IU), tetracycline (30μg), sulphamethoxazole (25 μg), chloramphenicol (30μg), gentamicin (10μg), ciprofloxacin (5μg), nalidixic acid (30μg), amoxicillin (10μg), ceftriaxone (30μg) and kanamycin (30 μg). All media and antibiotics used were Oxoid, UK products.

e) Quality control

Pre-tested questionnaire guided interview was used for data collection on socio-demographic characteristics. Specimens were collected and processed according to the standard operating procedure. Sterility of culture media was checked by incubating 5% of the batch at 35-37°c overnight and observed for bacterial growth and the standard reference strains, *S aureus* ATCC25923 (16) was tested weekly as controls on the biochemical tests and agar plates including Mueller Hinton agar with antimicrobial discs to assure testing performance of the potency of antimicrobial discs.

f) Data processing and analysis

Data was checked for its completeness and entered and analyzed using SPSS statistical software version 16.0. Results were explained in words and tables. Proportions for categorical variables were compared using chi-square test. In all cases P-value less than 0.05 was taken as statistically significant.

g) Ethical consideration

The project was started after ethical clearance was obtained from the Ethical Clearance Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Written informed consent was obtained from the study participants. Permission was obtained from Dessie Referral Hospital. For each confirmed infection cases, the responsible clinician of the participant was informed and treatment was started as per the culture result and antimicrobial susceptibility pattern. Confidentiality of information of the participants was maintained.

VI. Results

a) Prevalence of S. aureus infection in inpatients, nasal carriage of health personnel and hospital objects

Of 180 specimens collected, 40(22.2%) were from inpatients, 35(19.4%) from health personnel and 105(58.3%) from hospital objects. From 40 inpatients, 36(90%) had undergone surgery and developed hospital acquired wound infections and the other 4 (10%) were blood samples. A total of 73 *S. aureus* isolates were identified and of which, 23(31.5%), 14(19.2%), and 36(49.3%) were from inpatients, health personnel and objects respectively(table1)..

Table1 : Prevalence of *S. aureus* infection in inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Study participants	*S. aureus* status		
	Positive (%)	Negative (%)	Total (%)
Inpatients	23(57.5)	17(42.5)	40(22.2)
Health personnel	14 (40)	21 (60)	35 (19.4)
Hospital objects	36 (34.3)	69 (65.7)	105(58.3)
Total	73 (40.5)	107 (59.5)	180 (100)

As it is shown in table2, among 36 *S. aureus* isolates of objects, 13 were from blankets, 10 from cupboards and 13 from floor. Based on this finding, there was no significant difference (p=0.684) among these objects on the prevalence of *S. aureus*.

Table 2 : *S. aureus* isolates from objects* at Dessie Referral Hospital, May 2013

Variable	*S. aureus* status			
	Positive	Negative	Total	P-value
Hospital objects				0.684
Blankets	13 (36%)	22 (64%)	35 (33.3%)	
Cupboards	10 (27.7%)	25 (72.3%)	35 (33.3)	
Floor	13 (36%)	22 (64%)	35 (33.3%)	
Total	36 (34.3%)	69 (65.7%)	105 (100%)	

**blankets, cupboards and floor*

b) S. aureus infection in relation to sex and age groups of inpatients and health personnel

As presented in table 3, a total of 75: inpatients (40) and health personnel (35) had participated in the study. Of these, 45(60%) were females. Among 40 inpatients, 22 were females. From males, 61.1% and from females, 54.5% had *S. aureus* infection. Among 35 health personnel, 23 were females.

Table 3 : *S. aureus* infection in relation to sex and age groups of inpatients and health personnel at Dessie Referral Hospital, May 2013

Sex	Inpatients			Health personnel		
	Positive (%)	Negative (%)	Total (%)	Positive (%)	Negative (%)	Total (%)
Male	11 (61.1)	7 (38.9)	18 (45)	2 (16.7)	10 (28.6)	12 (34.3)
Female	12 (54.5)	10(45.5)	22 (55)	12(83.3)	11 (31.4)	23(65.7)
Total	23(57.5)	17(42.5)	40(100)	14 (40)	21(60)	35 (100)
Agegroup (year)						
0-10	0 (0)	2 (100)	2 (5)	0 (0)	0 (0)	0 (0)
11-20	2 (66.7)	1 (33.3)	3 (7.5)	0 (0)	0 (0)	0 (0)
21-30	10 (60)	7 (40)	17 (42.5)	5 (31.2)	11 (68.8)	16 (45.7)
31-40	4 (50)	4 (50)	8 (22.9)	4 (57.1)	3 (53.9)	7 (20)
≥41	7 (75)	3 (25)	10 (28.6)	5 (41.7)	7 (58.3)	12 (34.3)

c) Antimicrobial susceptibility pattern of S. aureus infection

Antimicrobial resistance patterns of *S. aureus* infection was 43.8%, 0%, 90.4%, 38.4%, 45.2%, 34.2%, 6.8%, 37%, 93.2%, 83.6%, 47.9%, and 35.6% for oxacillin, vancomycin, penicillin G, tetracycline, sulphamethoxazole, chloramphenicol, gentamicin, ciprofloxacin, nalidixic acid, amoxicillin, ceftriaxone, and kanamycin respectively. High level of resistance was demonstrated to penicillin G (90.4%), nalidixic acid (93.2%), and amoxicillin (82.9%), whereas, gentamicin (84.3%)), tetracycline (62.9%) chloramphenicol (63.6%), ciprofloxacin (61.6%), and kanamycin (64.4%) were relatively sensitive to *S. aureus* infection. Vancomycin exhibited 100% susceptible in all study participants (table4).

Table 4 : Antimicrobial susceptibility patterns of all *S. aureus* isolates (n=73) from inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Antimicrobial agents	Antimicrobial susceptibility patterns			
	Susceptible	Resistance	Intermediate	Total
Oxacillin	41(56.2%)	32 (43.8%)	0(0%)	73(100%)
Vancomycin	73(100%)	0 (0%)	0(0%)	73(100%)
penicillin G	6(8.6%)	66 (90%)	1(1.4%)	73(100%)
Tetracycline	45(62.9%)	28(37.1%)	0(0%)	73(100%)
Sulphamethoxazole	35(47.1%)	33(45.7%)	5(7.1%)	73(100%)
Chloramphenicol	47(62.9%)	25(35.7%)	1(1.4%)	73(100%)
Gentamicin	62(84.3%)	5(7.1%)	6(8.6%)	73(100%)
Ciprofloxacin	45(62.9%)	27(35.7%)	1(1.4%)	73(100%)
Nalidixic acid	1(1.4%)	68(92.9%)	4(5.7%)	73(100%)
Amoxicillin	10(14.3%)	61(82.9%)	2(2.9%)	73(100%)
Ceftriaxone	34(48.6)%	35(47.1%)	4(4.3%)	73(100%)
kanamycin	47(62.9%)	26(37.9%)	0(0%)	73(100%)

Different antimicrobials showed different antimicrobial susceptibility patterns in different study participants. Resistance pattern of isolates for nalidixic acid (91.3%), penicillin G(100%) and amoxicillin (100 %) were demonstrated in inpatient, whereas, in health personnel, the level of resistance were 85.7% for nalidixic acid, 92.9% penicillin G and 78.6% amoxicillin. In objects, the level of resistance for nalidixic acid, penicillin G and amoxicillin were 97.2% 83.3% and 75% respectively (table5).

Table 5 : Antimicrobial susceptibility patterns of *S. aureus* isolates from inpatients, health personnel and objects at Dessie Referral Hospital, May 2013

Antimicrobial agents	Study participants and antimicrobial susceptibility patterns								
	Inpatients			Health personnel			Objects		
	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)	S (%)	R (%)	I (%)
Oxacillin	14(60.9)	9(39.1)	0(0)	11(78.6)	3(21.4)	0(0)	16(44.4)	20(55.6)	0(0)
Vancomycin	23(100)	0(0)	0(0)	14(100)	0(0)	0(0)	36(100)	0(0)	0(0)
penicillin G	0(0)	23(100)	0(0)	1(7.1)	13(92.9)	0(0)	5(13.9)	30(83.3)	1(1.4)
Tetracycline	16(69.6)	7(30.4)	0(0)	7(50)	7(50)	0(0)	22(61.1)	14(38.9)	0(0)
Sulphamethoxazole	12(52.2)	9(39.1)	2(8.7)	8(57.1)	5(35.7)	1(7.1)	15(41.7)	19(52)	2(5.6)
Chloramphenicol	16(69.6)	6(26.1)	1(4.3)	12(85.7)	2(14.3)	0(0)	19(52.8)	17(47.2)	0(0)
Gentamicin	22(95.7)	0(0)	1(4.3)	14(100)	0(0)	0(0)	26(72.2)	5(13.9)	5(13.9)
Ciprofloxacin	15(65.2)	8(34.8)	0(0)	10(71)	3(21.4)	1(7.1)	20(55.5)	16(44.5)	0(0)
Nalidixic acid	0(0)	21(91.3)	2(8.7)	0(0)	12(85.7)	2(14.3)	1(2.8)	35(97.2)	0(0)
Amoxicillin	0(0)	23(100)	0(0)	2(14.3)	11(78.6)	1(7.1)	8(22.2)	27(75)	1(2.8)
Ceftriaxone	10(43.5)	10(43.5)	3(13)	10(71.4)	3(21.4)	1(7.1)	14(38.9)	22(61.1)	0(0)
kanamycin	18(78.3)	5(21.7)	0(0)	10(71.4)	4(28.6)	0(0)	19(52.8)	17(47.2)	0(0)

S= susceptible R= resistance I= intermediate

d) Multi drug resistance pattern of S. aureus isolates from inpatients, heath personnel and objects

Multi-drug resistance (resistance to ≥2 drugs) was recorded in 79 (95.9 %) of *S. aureus* isolates. About half, 39(53.4%) of the isolates were demonstrated resistant to at least five antibacterials. Four (5.5%), 2 (2.7%), 17 (23.3%) and 11(15.1%) of the *S. aureus* were found to be resistant for one, two, three and four antibacterials respectively. None of the *S. aureus* isolates were susceptible for all tested antibacterials (table6).

Table 6 : Multi drug resistance pattern of *S. aureus* isolates from inpatients, health personnel and objects, at DRH*, May 2013

Study participants	Antibiogram pattern						Total
	R0	R1	R2	R3	R4	≥R5	
Patients	0(0%)	0(0%)	1(4.3%)	5(21.7%)	4(17.4%)	13(56.5%)	23(31.5%)
Health personnel	0(0%)	0(0%)	1(7.1)	5(35.7%)	3(21.4%)	5(35.7%)	14(19.2%)
Objects	0(0%)	4(11.1%)	0(0%)	7(19.4%)	4(11.1%)	21(58.3%)	36(49.3%)
Total	0(0%)	4(5.5%)	2(2.7%)	17(23.3%)	11(15.1%)	39(53.4%)	73(100%)

R0= resistant to zero antimicrobial
R1= resistant to one antimicrobial
R2= resistant to two antimicrobial
R3= resistant to three antimicrobial
R4= resistant to four antimicrobial
R≥5= resistant to greater or equals to five antimicrobial
*Dessie referral hospital

VII. Discussion

Results of previous studies which are also confirmed in this study had shown that *S. aureus* is the common cause of nosocomial infection. Overall prevalence of *S. aureus* infection in this study (table1) is comparable to other study done elsewhere in the world (37.3%) (17). The present study also showed that the frequency of *S. aureus* isolated from hospital objects of different wards (table2) is consistent with studies done in Gondar and Nigeria (17,18).

One of the important sources of *S. aureus* for nosocomial infection is nasal carriage among hospital personnel (19). In this study, prevalence of *S. aureus* isolates from nasal carriage of health personnel and hospital objects (table1) are comparable with other studies done in Gondar, Pakistan and Cameron (17, 20, 21). The occurrence of *S. aureus* in hospital objects (table2) may indicate poor infection control in the hospital environment, which could serve as a reservoir of the organism and it may be the potential source of cross contamination (infection) between objects and

patients. This may also account for the high incidence of the organism observed in health personnel. Out of 50 isolates of *S. aureus* from health personnel and objects, 19 had identical antibiogram pattern with the isolates of patients. This specifies that the objects and/or the health personnel may be the source of *S. aureus* infection in this study.

Antimicrobial resistance patterns of *S. aureus* infection in the present study (table4) is comparable with the previous study done in Dessie (22), but the susceptibility of ciprofloxacin and ceftriaxone are fall from the previous study which had such antimicrobial susceptibility patterns as 95.4% and 80% respectively. It may be due to overuse of it as empirical treatment.

S. aureus isolated in this study showed the highest vancomycin sensitivity pattern (table4) which is similar with the previous studies in Kontagora and Suleja Area of Niger State, in Gondar and Nigeria (17, 23, 24) The highest susceptibility of *S. aureus* to vancomycin in our study may be due to its uncommon use or being a new medication. In this study; however, *S aureus* was highly resistant to penicillin G, amoxicillin and nalidixic acid (table 4). This result is in line with previous studies in Gondar, Cameron, Dessie and Jimma (24, 25, 28, 25), respectively, but in the case of amoxicillin, our result is completely showed disparity to the study in Bahar-dar (26), which reported *S. aureus* as 100% susceptible. This difference may be due to inappropriate and incorrect administration of antibacterials as empiric therapies and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organism in the community in the study area. Forty four percent of *S. aureus* isolates were resistant to oxacillin which is similar to the previous studies in Kontagora and Suleja Area of Niger State and Jimma (23, 25).

Multi drug resistance patterns (table 6) of isolates of *S. aureus* in the current study is higher than the previous studies in Gondar (17) and Dessie (22) but in line with the previous study in Gondar (27). This is probably due to empirical use of broad-spectrum antibacterials, lack of culture and antimicrobial susceptibility tests and non adherence to hospital antimicrobial policy. About 24%, 16%, 6%, and 3% of *S. aureus* isolates were found to be resistant to three, four, two and one of the tested antibacterials respectively. No one of the isolates was susceptible to all of the tested antibacterials and also none of the *S. aureus* isolates were pan- resistant (resistant to all the tested antibacterialsl).

VIII. Limitation of the Study

In the present study, the antimicrobial susceptibility pattern was used in an attempt to identify possible cross infection from health personnel and/or hospital objects has a limitation. Since unrelated colony of a single species can undergo evolutionary convergence to the same resistance phenotype under antibacterial selective pressure through mutation and genetic exchange (28), unless confirmed by genomic analysis, no definite conclusions can be drawn with regard to the role of the possible sources of infection.

IX. Conclusion

The present study indicated that *S. aureus* is still the most common cause of nosocomial infection and hospital objects which were being used by inpatients may be a source of nosocomial *S. aureus* infections in this hospital. It also demonstrated that health personnel are at risk of the infection and can be a potential source of nosocomial *S. aureus* infections. In this study MDR was very high and most of the isolates showed high levels of resistance to commonly used antibacterials. However, gentamicin (84%) had high activity against most of the isolates *in vitro* when compare to other tested antibacterials. Susceptibility rate of *S. aureus* to vacomycin in this study was 100%. In the absence of culture and antibacterial susceptibility testing, vancomycin and gentamicin are the best therapeutic options to treat *S. aureus* infections. In order to confirm *S. aureus* cross infections among patients, health personnel and objects, further study with the aid of phage typing and other molecular studies should be done.

X. Acknowledgment

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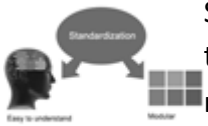

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- Not at all, take in raw data or intermediate calculations in a research manuscript.
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- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

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Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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