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Survey on Antibiotic usage

Highlights

Primary Tuberculosis of Tongue

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Evaluation of Factors Regarding Misuse of Antimicrobials in Poultry and Piggery Farms in Abia State, Nigeria

By N. Amaechi

Michael Okpara University, Nigeria

Abstract- A survey was conducted to evaluate the factors that can cause misuse or abuse of antimicrobials in poultry and piggery farms. This evaluation was conducted between May 2011 and April, 2012 using a structured questionnaire distributed by the author to farm managers. The results showed that level of education of farm managers have a role to play in prudent use of antimicrobials. Only a small percentage (10% for poultry, 20% for piggery farms) of antimicrobial prescriptions were made by veterinarian, while the key prescriptions (70% poultry, 60% piggery farms) were made by the farmers or the farm managers. Laboratory analysis was not routinely done on samples before antimicrobial administration. Majority of antimicrobial administration (45% for poultry farms, 60% for piggery farms) were based on history of disease and mere observations on the animals. In the poultry farms, 60% of farm managers have degree and they adhered to the average of 3-4 days (65%) duration of antimicrobial administration. The results showed that majority of antimicrobial administrations were made by farm managers with minimal laboratory analysis of samples. It is anticipated that this findings will create awareness on the appropriate use of antimicrobials on farm animals.

Keywords: antimicrobial misuse, poultry farms, piggery farms, evaluations.

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Evaluation of Factors Regarding Misuse of Antimicrobials in Poultry and Piggery Farms in Abia State, Nigeria

N. Amaechi

Abstract- A survey was conducted to evaluate the factors that can cause misuse or abuse of antimicrobials in poultry and piggery farms. This evaluation was conducted between May 2011 and April, 2012 using a structured questionnaire distributed by the author to farm managers. The results showed that level of education of farm managers have a role to play in prudent use of antimicrobials. Only a small percentage (10% for poultry, 20% for piggery farms) of antimicrobial prescriptions were made by veterinarian, while the key prescriptions (70% poultry, 60% piggery farms) were made by the farmers or the farm managers. Laboratory analysis was not routinely done on samples before antimicrobial administration. Majority of antimicrobial administration (45% for poultry farms, 60% for piggery farms) were based on history of disease and mere observations on the animals. In the poultry farms, 60% of farm managers have degree and they adhered to the average of 3-4 days (65%) duration of antimicrobial administration. The results showed that majority of antimicrobial administrations were made by farm managers with minimal laboratory analysis of samples. It is anticipated that this findings will create awareness on the appropriate use of antimicrobials on farm animals.

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I. INTRODUCTION

The use of antimicrobial compounds in food animals production provides demonstrated benefits, including improved animal health, higher production and in some cases, reduction in food borne pathogens. For many decades, antimicrobial resistance has been recognized as a global health problem (CDC, 2010). Some of its causes are widely accepted, for example, the overuse and inappropriate use of antimicrobial for non bacterial infections and inadequate antimicrobial stewardship in the clinical arena. Some of the types of antimicrobial misuse in clinical practice include unjustified prescription, under prescription, under dosing and short duration (Bartoloni *et al*, 1998). Antimicrobial prescription in many developing countries is almost entirely empirical and based on surveillance data obtained from locations or at a time that it is unlikely to be relevant to the ensuring situation.

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Another problem is the practice of farmers who are more likely to fail to complete a prescribed course of antimicrobials (Pechere, 2001). Antimicrobial choice is based on experience, folklore or customer preference usually in complete disregard of the long-term consequences of antimicrobial uses. Clearly, factors like cost and availability of diagnostic/health care influences antimicrobial misuse.

Priority should be given to education, directed at prescribers of antimicrobial to reduce inappropriate use (O'Connor *et al*, 2001). Despite the widespread adoption of antimicrobial use in food animals, reliable data about the quantity and patterns of use (e.g. dose and frequency) are not available (Surmah *et al*, 2006). Quantifying antimicrobial use in food animals is challenging due to variations in study objectives- investigators may measure only therapeutic uses, only non therapeutic uses, or a combination thereof, depending on their outcome of interest- and lack of clarity surrounding the definitions of therapeutic vs non therapeutics.

As a result of the aforementioned problems, there is need to study and evaluate the factors that necessitated the misuse of antimicrobials in poultry farms and commercial piggery farms in Abia State, Nigeria.

II. MATERIALS AND METHODS

a) Animal production

A total of 90 poultry and 72 piggery farms from 17 local government areas of Abia State, Nigeria were solicited to participate in the evaluation of misuse of antimicrobials. The poultry and piggery farms that participated in this study were recommended by Agricultural Development Project (ADP) officials in Abia State and were classified as large poultry farms and commercial piggery farms respectively. Poultry and piggery producers were selected for the study if they:

1. Would allow access to available farm records related to antibiotic use
2. Granted permission to interview them on antibiotic use
3. Possess records that relates to morbidity, mortality, education levels of their workers and contacts with veterinarians. The 70 poultry and 60 piggery farms

that met the study criteria were requested to take part in the evaluation.

b) Survey Questionnaire

Well-structured questionnaires on antimicrobial usage were administered by the author to poultry and piggery producers or managers of each farm. The first part of the evaluation questionnaire was administered to all 70 poultry farms. The second part of the evaluation questionnaire was administered to all 60 piggery farms. This evaluation was conducted from May 2011 to April, 2012.

c) Statistical Analysis

Answers to the questionnaire were analyzed using regression analysis to determine the factors that

are responsible for abuse or misuse of antimicrobials. Also simple statistical methods such as mean, bar chart and percentages were used to interpret the questionnaires. All analysis was done using SPSS (2006) version 11.5 software.

III. RESULTS

Figure 1 depicts the level of education of poultry and piggery farmers. Among the poultry farmers, degree holders occupied the highest percentage (60%), while for piggery farmers, secondary school certificate (SSCE) "O" level holders had the highest percentage (35%).

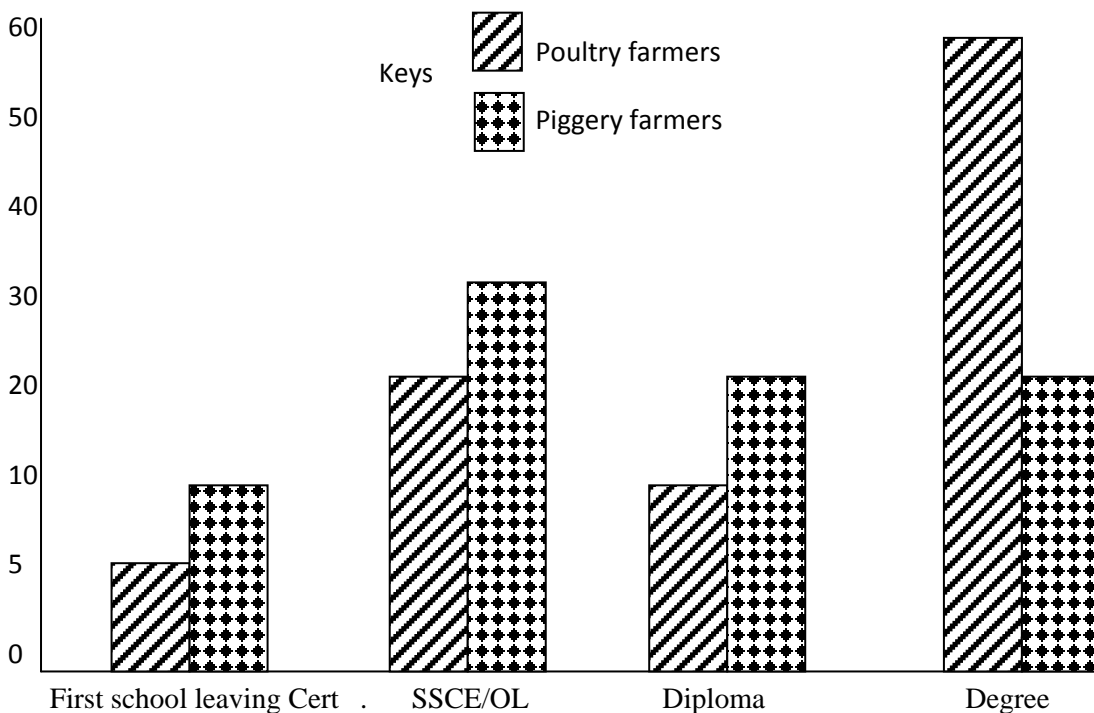


Figure 1 : Distribution of poultry and piggery workers according to their level of education

The duration of antimicrobial administration was investigated to ascertain if farmers adheres to the duration of drug administration. Figure 2 showed that majority of the antimicrobials were given within 3-4 days (65%) in poultry farms and (60%) in piggery farms.

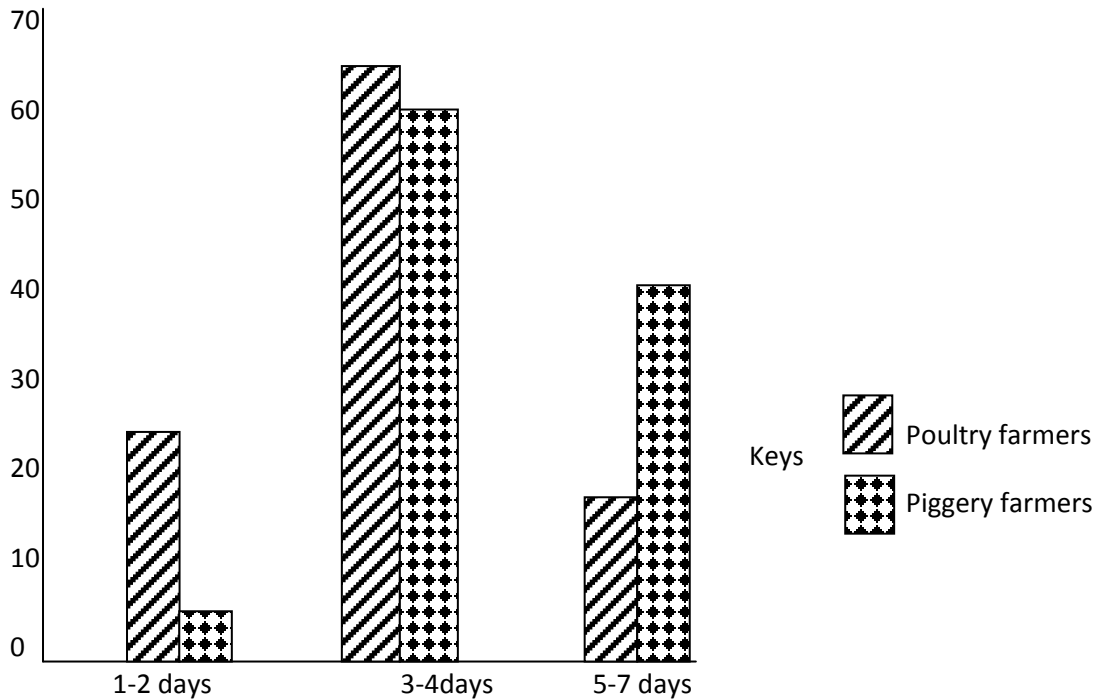


Figure 2: Duration of antimicrobial administration in poultry and piggery farms

The tendency to rely on personal experience for antimicrobial use, dosage and withdrawal period inspired the researcher to investigate on personnel that prescribe antimicrobial agents.

while in piggery farms majority of drug prescriptions were made by farmers (60%). In both poultry and piggery production, farmers made the highest number of prescriptions.

Figure 3 showed personnel that makes prescription of antimicrobial administration. In poultry farms, 70% of drug prescription was made by farmers,

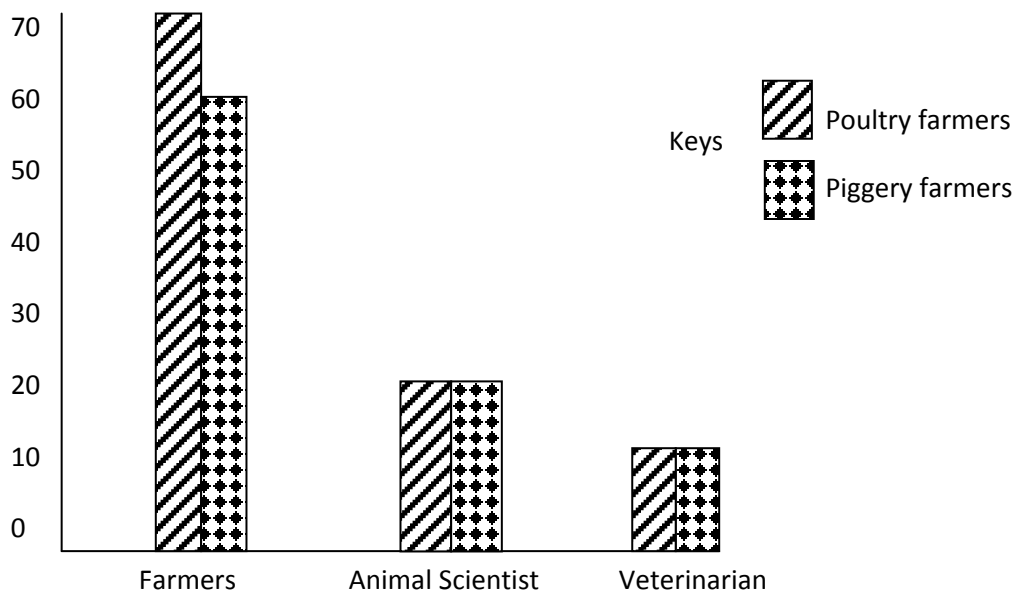


Figure 3: Personnel that makes prescription of antimicrobial administration

Figure 4 showed that majority of antimicrobials used were given to pigs and poultry birds without laboratory analysis done on samples to isolate aetiological agents. In poultry farms, 65% had no laboratory analysis done on samples prior to antimicrobial use, while it was 75% in piggery farms.

farmers use antimicrobials in treatment. For the poultry workers clinical investigation (50%) accounts for an important reason for treatment using antimicrobials. For the piggery farmers history of disease and mere observation account for 60% of their reasons for treatment.

The basis for treatments using antimicrobials was investigated. Figure 5 showed the basis why

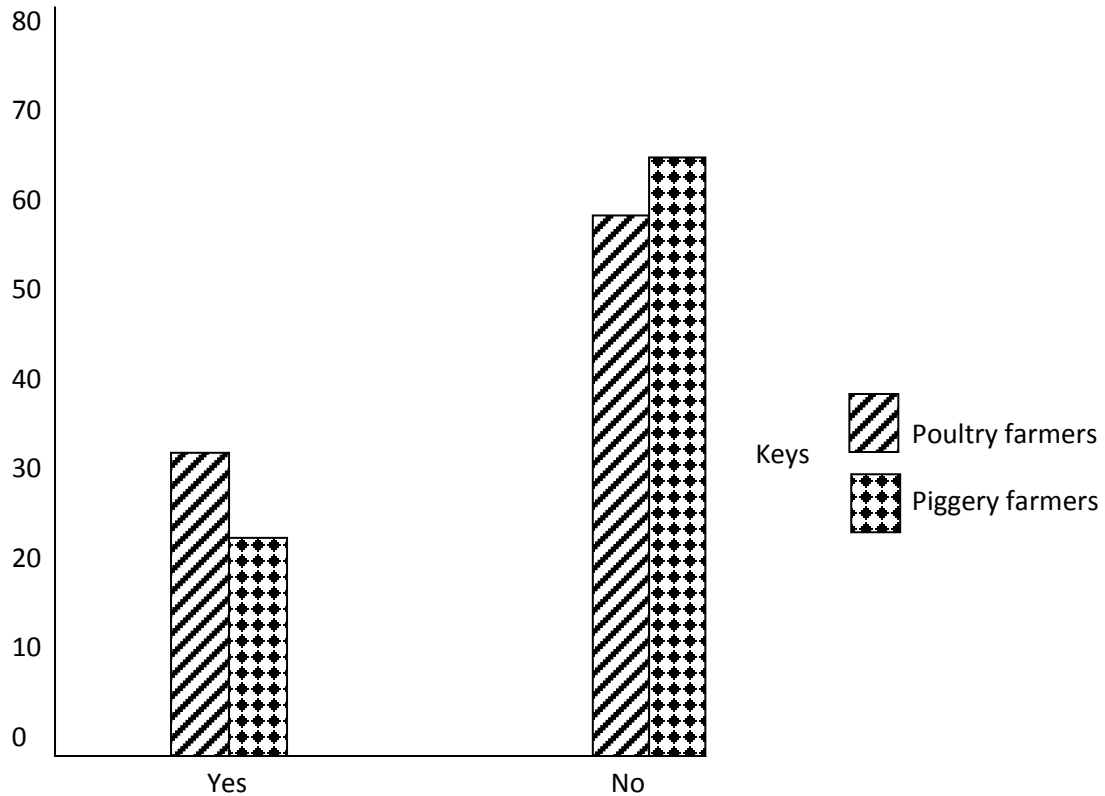


Figure 4 : Any laboratory analysis done prior to antimicrobial use?

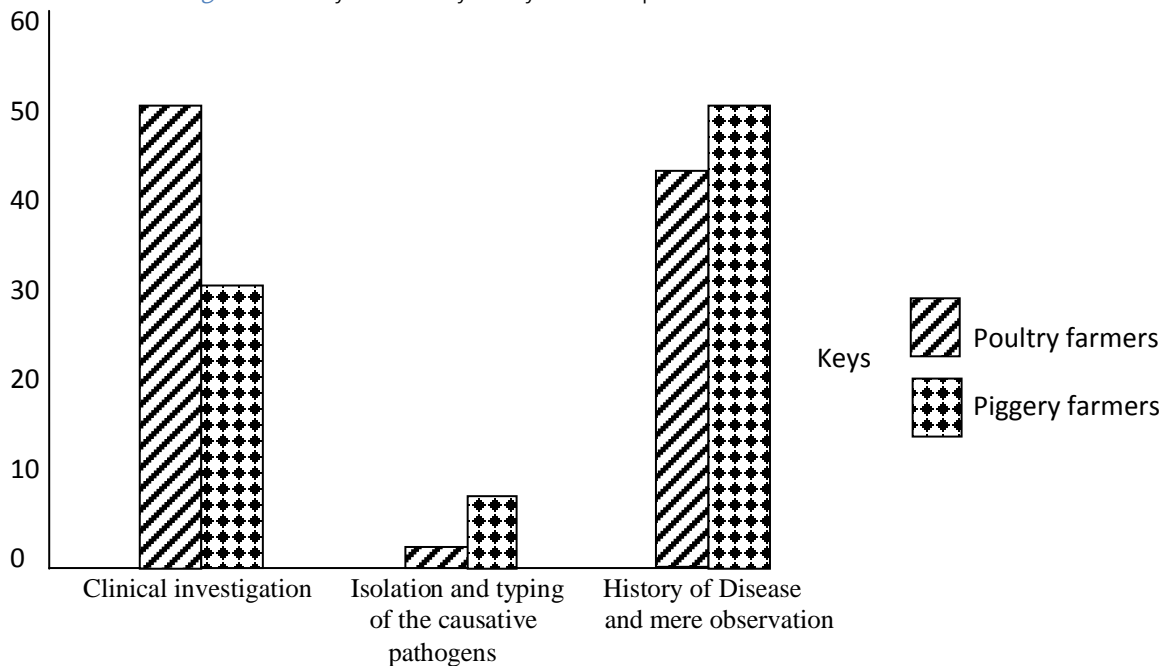


Figure 5 : Basis for treatment using antimicrobials on poultry birds and pigs

Response of respondents on whether the antimicrobial treatment was completed or not is shown in Figure 6. In the poultry farms, 70% of farmers affirmed

that the antimicrobial treatment was completed according to the prescription and duration of treatment, while in the piggery farms, it was 60% of the farms.

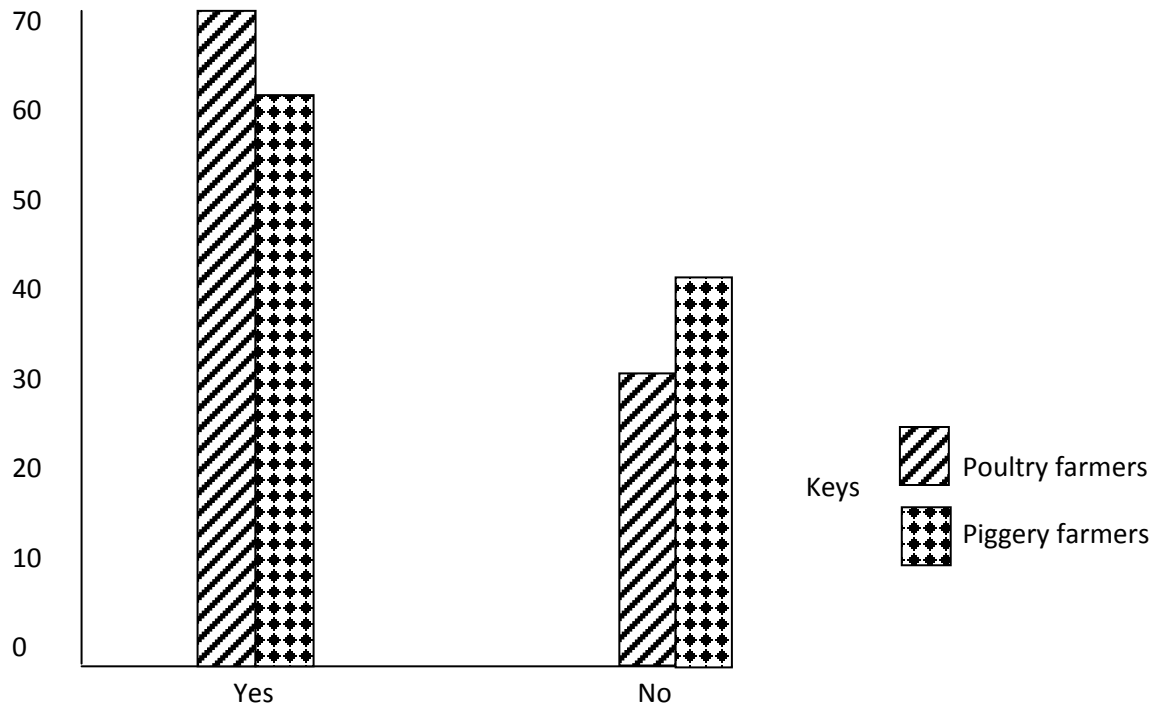


Figure 6 : Completion of antimicrobial treatments in poultry birds and pigs

Figure 7 which showed the frequency of consulting a veterinarian indicated that in poultry farms veterinarians were frequently (40%) consulted, while in piggery farms veterinarians were mostly consulted during emergency (40%). These differences in response in the frequency of consulting veterinarian among the poultry and piggery farmers might be as a result of acute nature of some poultry diseases as opposed to

pig diseases. Also since majority of piggery farmers are SSCE O/Level holders who may not see any reasons of consulting veterinarians except during emergency. This is in contrast to poultry workers where majority of their workers are educated (degree holders), who sees the need for consulting veterinarian frequently for advices and treatments.

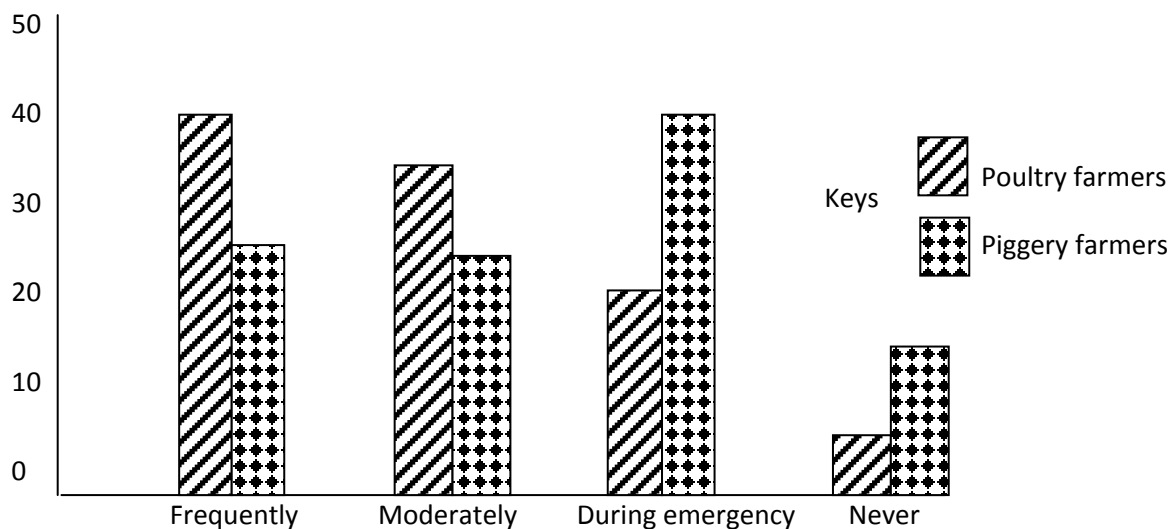


Figure 7 : Frequency of consulting a veterinarian

IV. DISCUSSIONS

Antibiotics are widely used in food animal production for therapy and prevention of bacterial infections and for growth promotion (McEwen and Fedorka-Cray, 2002). Food animals are raised in confined conditions that promote the spread of infectious diseases (WHO, 1997). Most enlightened farmers are involved in poultry business because it involves the use of intellectual and experience. Because of the vulnerability of the poultry birds, extra carefulness and knowledge are needed for the business to thrive well. This was not the same with piggery farming. Pigs which are omnivores thrive well on any type of food including kitchen wastes, garbage etc. This might be the reason why educated and non-educated were involved in the pig production as can be seen in Figure 1.

Antimicrobials are often over used because of low cost and ready availability, often without veterinary prescriptions and are administered for long periods of time at sub-therapeutic concentrations to entire groups or herds of animals (Gossens, 2009). Sixty-five percent of antimicrobials were used in poultry farms visited without laboratory analysis, while 75% of antimicrobials were used in piggery farms without laboratory analysis. Lack of diagnostic services (65% for poultry, 50% for piggery farms) was the major reason given by farmers for not carrying out laboratory diagnosis on samples. This might be due to unavailability of equipment and materials for this service or the cost of this service may hinder farmers with meager income to pay for such analysis. The over-use of antimicrobials in intensive pig farming is implicated in the emergence of a new pig strain of the superbug methicillin-resistant *Staphylococcus aureus* (MRSA) (Wease and Van Dugkeren, 2010).

The antimicrobials were administered based on clinical investigation in poultry birds (50%) and history of disease and mere observation in pigs (60%). This showed that majority of treatments were not based on isolation and typing of causative pathogens. It means that diagnoses were done tentatively instead of confirmatory. Resistance has developed to virtually all antimicrobials used in food animals. The most important driver of resistance selection and spread is antibiotic over-use (Aarestrup *et al*, 2008; O'Brien, 2002). Causes of abuse or misuse of antimicrobials in food animals showed that education on antimicrobial resistance was lacking amongst dispensers and prescriber of antimicrobials. Records from questionnaire also showed that other causes of abuse of antimicrobials in food animals included that prudent antimicrobial use was lacking amongst dispensers and prescribers of antimicrobials; inappropriate doses were frequently used in food animals. Also included are purchase and administration of antimicrobials without proper prescription. These were done ignorantly not minding

the availability of veterinarian. Other factors identified from the study included inefficient regulatory mechanism on the use of antimicrobials, marketing of substandard antimicrobials and inappropriate doses of drugs frequently used in food animals.

It is suggested that the extensive and often indiscriminate use of antimicrobial agents in animal husbandry might constitute a risk factor in creating an animal reservoir of antimicrobial-resistance bacteria (Tenover, 2001). From this reservoir, resistant strains or resistance genes might spread to humans via the food chain. Some data have indicated that food-producing animal species, raw poultry and other meat products harbor antimicrobial-resistant, Gram positive bacteria (Aarestrup *et al*, 2008). The increased global flow of antimicrobials brought with it the threat of antimicrobial resistance.

The tendency to rely on history of disease and mere observation for antimicrobial use, dosage and withdrawal period was observed among the farmers. These lapses could lead to improper antimicrobial usage as was observed by Zwald *et al*, (2004) in dairy farms. Absence of antimicrobial treatment records, the lack of written plans for treating sick animals, the failure to complete an antimicrobial treatment course were other factors that could lead to inappropriate use of antimicrobials and emergence of antimicrobial resistant bacteria.

V. CONCLUSION

Antimicrobial abuse is the main driver in the issue of antimicrobial resistance. The misuse and abuse of antimicrobials in food animals is a major source of the problem. Improved surveillance and national regulation is needed to ensure that antimicrobials are used prudently and are not routinely fed to animals for non therapeutic purposes. National authorities, veterinarians, physicians and farmers all have a role in "preserving the power of antibiotics".

VI. RECOMMENDATION

Antimicrobial use data for animals should be made available to aid in assessing the public health impacts of antimicrobial use in animals and policy changes on antimicrobial consumption.

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Primary Tuberculosis of Tongue; Mimicking as a Malignancy- A Case Report

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Abstract- Though tuberculosis in India is very common but tuberculosis of oral cavity is very rare. It is prevalent as 0.5 to 5% of all cases¹. Integrity of the oral epithelium and inhibitory effect of saliva are considered to be the reason for relative resistance to infection of bacilli,² hence it is low prevalent. Though its a case of primary tuberculosis of tongue giving suspicion of malignancy in an otherwise healthy elder male belonging to low socioeconomic class.

Keywords: *tuberculosis, granuloma, malignancy, ulcer.*

GJMR-C Classification : *NLMC Code: WA 400*



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Primary Tuberculosis of Tongue; Mimicking as a Malignancy-A Case Report

Tomar K.P.S ^α, Pandit Vidyanand ^σ & Jain Bharat ^ρ

Abstract- Though tuberculosis in India is very common but tuberculosis of oral cavity is very rare. It is prevalent as 0.5 to 5% of all cases¹. Integrity of the oral epithelium and inhibitory effect of saliva are considered to be the reason for relative resistance to infection of bacilli,² hence it is low prevalent. Though its a case of primary tuberculosis of tongue giving suspicion of malignancy in an otherwise healthy elder male belonging to low socioeconomic class.

Keywords: tuberculosis, granuloma, malignancy, ulcer.

Abbreviation: HIV-human immunodeficiency virus, ATT-anti tubercular therapy.

I. INTRODUCTION

Tuberculosis of oral cavity is very rare. It is prevalent as 0.5 to 5% of all cases¹. Integrity of the oral epithelium and inhibitory effect of saliva are considered to be the significant reason for relative resistance to infection of bacilli². Out of 8.6 million cases of the world, 2.2 million (25%) cases occurred in India making India as the World's highest tubercular burden country. In July 2011 the revised estimated incidence was 185/lac, prevalence was 285/lac and mortality rate of T.B was 22/lac³. Though extra pulmonary tuberculosis only represents 15% of total cases (in HIV negative patients)⁴. Tuberculosis of oral cavity is an uncommon site for involvement however tongue is the most common site in oral cavity accounts almost half of its cases⁵. Here it is the presentation of a case report of a patient who have only primary tuberculosis of tongue no other systemic tuberculosis involvement & was healthy (not immunocompromised).

II. CASE REPORT

A 52 yr old male came to the department of otorhinolaryngology with the presenting complaints of erosive lesion over tongue with slight difficulty in chewing and speaking, though it was a painless lesion. Patient have a long term history of tobacco chewing approximately for 30yrs. No other relevant complain or history.

On general examination, patient was well build and no complains of cough or fever, both lungs were clear. There were no lymphadenopathy or organomegaly.

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Oral examination shows poor oral hygiene with stained gums and teeth and a erosive lesion over dorsum surface at anterior two third of tongue. FNAC was advised which was inconclusive. Haematological parameters were within normal limits. But due to suspicion of malignancy by surgeon and patients over concern hemiglossectomy was done and resected portion was sent for histopathological examination to the Pathology Department. Histopathological features showed chronic noncaseative granuloma formation with giant cells suggestive of tubercular involvement of tongue.

III. DISCUSSION

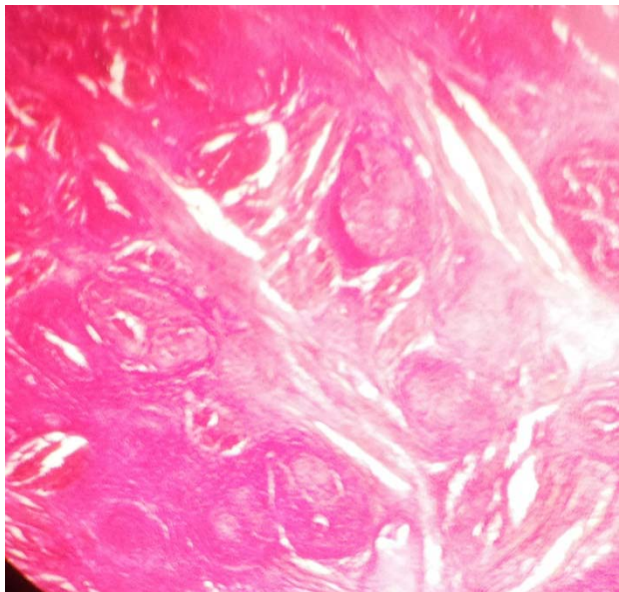
Tuberculosis involve almost every organ of body. Although involvement of tongue is a very rare. The first authentic case of this was reported by Portal⁶ in 1804 and Mogargani⁷ in 1761 described patient with tubercles in tongue. Pathogenesis behind this is recurrent contact with sputum or hematological spread. Majority of the cases belongs to the HIV infected peoples or immune compromised patients. Other sites of involvement in oral cavity are floor of mouth, soft palate gingiva lips & hard palate. Its common in middle aged or elder men⁸. These oral tubercular lesions generally develop secondary to pulmonary tuberculosis but occasionally primary involvement is seen in cases with poor oral hygiene and traumatic injury during dental or oral surgical procedures⁹. Titche listed five types of tongue involvement 1.ulcer 2.fissure 3.granuloma 4.tuberculoma 5.glossitis. and ulcer is the most common finding¹⁰. In the present case patient have tubercular granuloma with painless lesion. Treatment of extra pulmonary tuberculosis is comes under category II ATT. First line drugs to be given are rifampicin, isoniazid, ethambutol, pyrazinamide & streptomycin.

IV. CONCLUSION

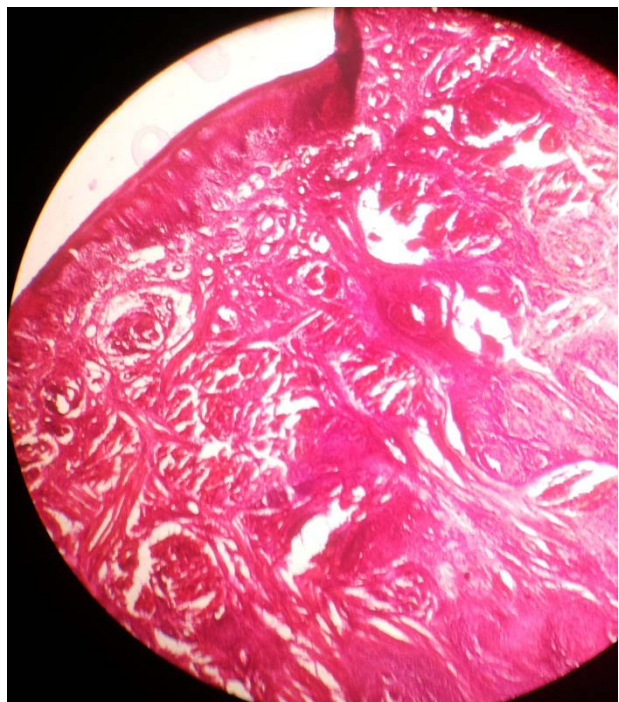
Though it is a very rare presentation of tuberculosis of tongue but clinician should consider always this in a differential diagnosis of the chronic ulcerative lesion of tongue. To rule out this a adequate biopsy from deeper and representative area should be taken before doing hemi or complete resection of tongue. This can avoid unnecessary operative procedure and disability to the patients as treatment with ATT very effective most of the time.

a) *Histopathological Sections*

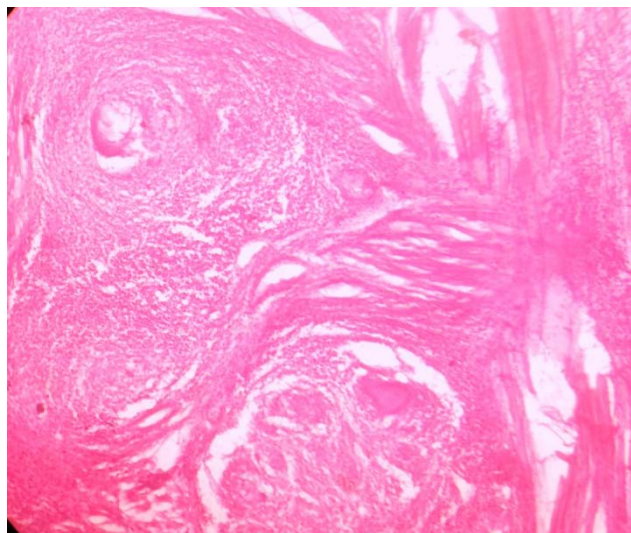
The Histopathological Sections are depicted in following different views of the present case.



scanner view (Tubercular Granuloma)



10x(Tubercular Granuloma)



40x(Tubercular Granuloma)

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A Survey on Antibiotic Usage in Pigs and Poultry Birds in Abia State, Nigeria

By N. Amaechi

Michael Okpara University, Nigeria

Abstract- A survey was conducted (June 2011 to May 2012) on antibiotic usage of 90 poultry farms and 72 piggery farms from 17 local government areas of Abia State, Nigeria. Survey questionnaires were used to identify and prioritize the antibiotics most effective and frequently used, current therapeutic and sub-therapeutic antibiotic usage patterns. The result showed that 65% of poultry and 75% of piggery farms failed to do laboratory analysis prior antibiotic usage and on most farms (70% for poultry, 65% for piggery) antibiotics were administered by the owner/manager. The mode of administration of the antibiotics was 80% through water in poultry and 80% through injection in piggery and 40% of the farmers said they always completed the course of antibiotic treatment. Twelve antibiotics including beta-lactams, streptomycin, tetracycline, macrolides, sulfa-drugs, cephalosporin etc. were used on these farms. These antibiotics were used mainly on weekly basis in poultry (65%) and fortnightly in piggery (40%). The result of this survey suggested that antibiotics are used extensively on poultry and piggery farms for therapeutics, prophylactic and growth purposes. Tetracycline and streptomycin were the most widely used antibiotics to treat mainly enteritis and pneumonia. There is a considerable variation in the management practices on poultry and piggery farms associated with antibiotic use. It is anticipated that the findings of this survey will help to develop new strategies for prudent use of antibiotics in piggery and poultry farms in Abia State, Nigeria.

Keywords: *survey, antibiotic use, poultry birds, pigs.*

GJMR-C Classification : *NLMC Code: QV 252, QV 269*



A SURVEY ON ANTIBIOTIC USAGE IN PIGS AND POULTRY BIRDS IN ABIA STATE, NIGERIA

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I. INTRODUCTION

The different applications of antibiotics in food animals have been described as therapeutic, prophylactics and sub-therapeutic uses. Therapeutic uses in clinically ill animals involve using curative dose in antibiotic agents for a relatively short period of time. In appropriate doses, empiric treatment and non-submission of clinical specimens and samples from sick animals are important contributing factors to antibiotics abuse (Smith *et al.*, 2005).

A study that examined the use of antibiotics on pigs and poultry birds reported that some drugs are abused especially tetracycline and streptomycin (Sawant *et al.*, 2005). Antibiotics usage varies from country to country, within a country and between farms, depending on policies and desired results. International, national and local antibiotic stewardship campaigns have been developed to encourage prudent use of and

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limit unnecessary exposure to antibiotics; with the ultimate goal of preserving their effectiveness for serious and life-threatening infections (Balongia *et al.*, 2005).

There is also considerable debate in Veterinary Medicine regarding use of antibiotics in animals raised for human consumption (food animals). An inherent consequence of exposure to antibiotic compounds, antibiotic resistance arises as a result of natural selection (Aminor and Mackie, 2007). The potential threat to human health resulting from inappropriate antibiotic use in food animals is significant, as pathogenic resistant organisms propagated in these food animals are poised to enter the food supply and could be widely disseminated in food product (Garafalo *et al.*, 2007 and Parveen *et al.*, 2007).

While antibiotic use in food animals may represent a risk to human health, the degree and relative impact have not been well characterized. Despite the wide spread adoption of antibiotic use in food animals, reliable data about the quantity and patterns of use (e.g. dose and frequency) are not available (Sarmah *et al.*, 2006). Quantity antibiotic use in food animals is challenging due to variations in study objectives - investigators may measure only therapeutic uses or a combination uses, only non-therapeutic uses or a combination thereof, depending on their outcome of interest. To understand the public health risks associated with antibiotic usage within pigs and poultry birds, it is important to define the type and specific use of antibiotic agents that are associated with on-farm management practices.

II. MATERIALS AND METHODS

a) Study participants

The study participants were drawn from poultry and piggery farms located in the study area. Poultry birds and pigs were the animals chosen because of the experienced farm managers. A total of 90 poultry and 72 piggery farms were solicited to participate in the antibiotic usage survey. The poultry farms classified as large poultry farms and the piggery farms classified as commercial piggery farms are scattered all over the seventeen (17) local government of Abia State.

b) Survey Questionnaire

What is the contribution of animal's sources to resistance among human pathogens? One approach to answer this question has been to direct questionnaires

to informed experts (Bywater, 2005). These questionnaires were developed to identify and prioritize the antibiotics most effective and frequently used by poultry and piggery farmers and to estimate the perceived contribution of antibiotic overuse (abuse) in animal agriculture to the occurrence of antibiotics resistant bacteria. The questionnaire also requested information about current therapeutic and sub-therapeutic antibiotic usage patterns and personnel data. The questionnaire survey was administered by the researcher to the senior farm managers, while records on drug usage and other management practice, were considered. The first part of the survey was administered to 60 commercial swine farms; the second part of the survey was administered to 60 commercial poultry farms that had records on herd/flock health and antibiotic use. The survey was conducted from May 2011 to April 2012.

c) Data Analyses

Answers to the questionnaire were analyzed using regression analysis to determine the factors responsible for abuse or misuse of antibiotics. Also simple statistical methods such as frequency, bar chart and percentage were used to interpret the questionnaires. All analyses were done using SPSS (2006) version 11.5 software.

III. RESULTS

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emerging of resistant bacteria (Gossens *et al.*, 2005). The resistance bacteria in animals due to antibiotic exposure can be transmitted to humans through consumption of meat, from close or direct contact with animals or through the environment (Schneider and Garrett, 2009). The survey conducted in this study included questions that were helpful to gain insight regarding farm management practices associated with antibiotic usage. One important finding of this study was the observation that the commonly used antibiotics in poultry farms was tetracycline (65%), in commercial pig farms was streptomycin (45%). Figure 1 showed that the use of tetracycline was significantly ($P < 0.05$) higher than other antibiotics used in poultry farms due to the availability of this drug over the counter.

In large-scale poultry production, antibiotics are usually administered through water. The weak and sick ones may not be able to get enough doses, while the stronger ones will get excess. Therefore, the mode of antibiotics administration was investigated.

Figure 2 showed that most of the drugs were administered through water (80%) in poultry farming, through injection (80%) in piggery farming.

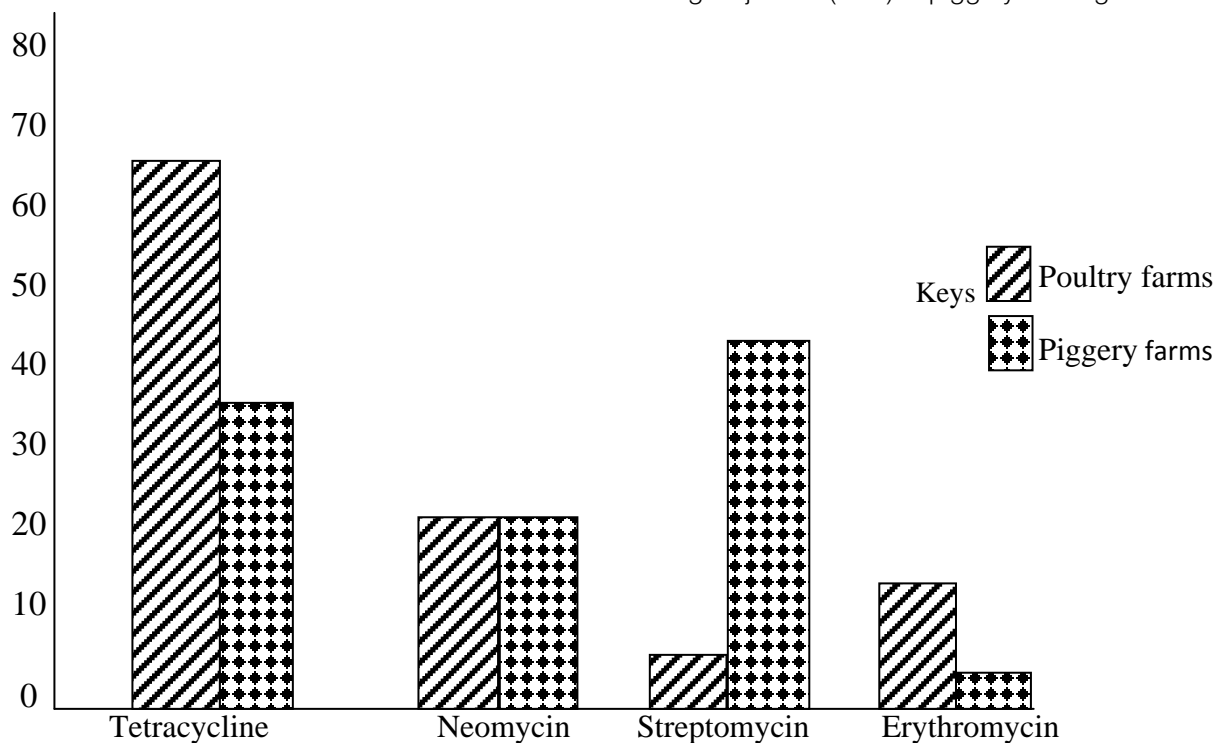


Figure 1: The most commonly used antibiotics in poultry and piggery farms

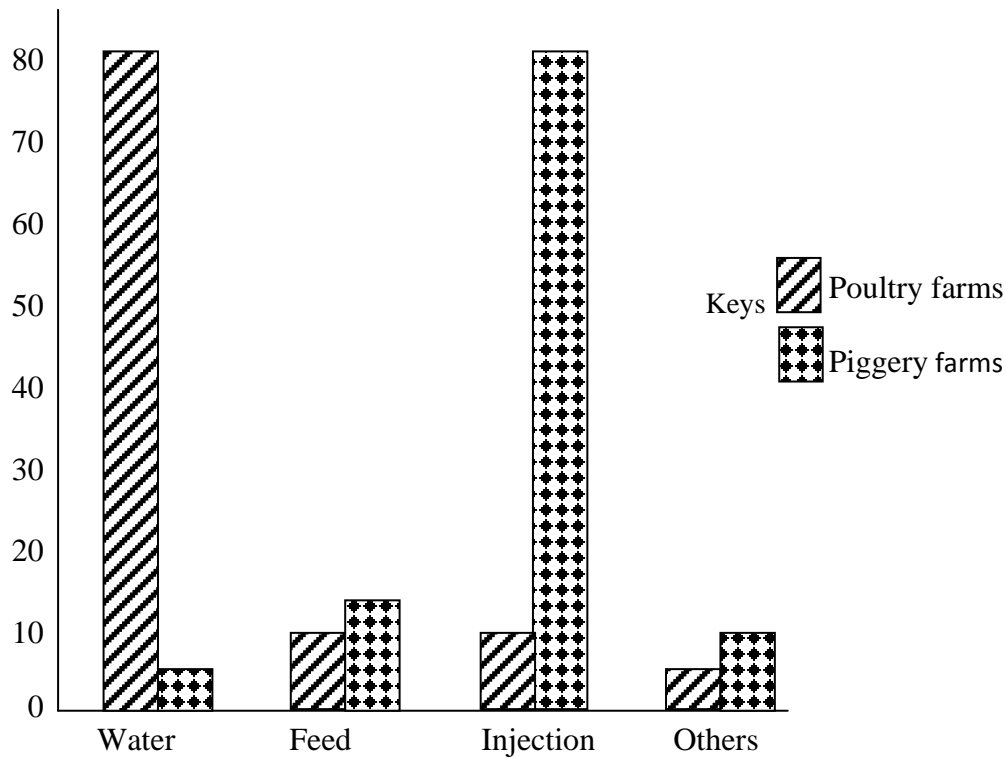


Figure 2: Modes of administration of antibiotics in poultry and piggery farms

Figure 3 showed that majority of antibiotic given was for treatment i.e. 60% for poultry birds and 65% for pigs. Figure 4 showed that majority of antibiotics used were given to pigs and poultry birds (75% and 65% respectively) without laboratory analysis done on samples to isolate aetiological agents. Administration of antibiotics without proper identification of aetiological agents leads to inappropriate treatment and development of resistant microorganisms. The lack of diagnostic services was the major reason why most farmers do not carry out laboratory diagnosis analysis of samples before administering antibiotics in poultry birds and pigs.

Figure 5 showed that antibiotics were more frequently used in poultry farms (weekly) than in piggery farms (fortnightly). This might be as a result of the more vulnerability of poultry to diseases than pigs. Figure 6 showed that personnel that make the prescription of antibiotics were the farmers/managers (70% in poultry, 65% in pigs). The tendency to rely on personal experience for antibiotic use, dosage and withdrawal period could lead to improper antibiotic usage. Responses of respondents showed that 80% of poultry farmers affirmed that the antibiotic treatment was completed according to the prescription and duration of treatment, while in piggery farms, it was 62% of farmers who affirm thus:

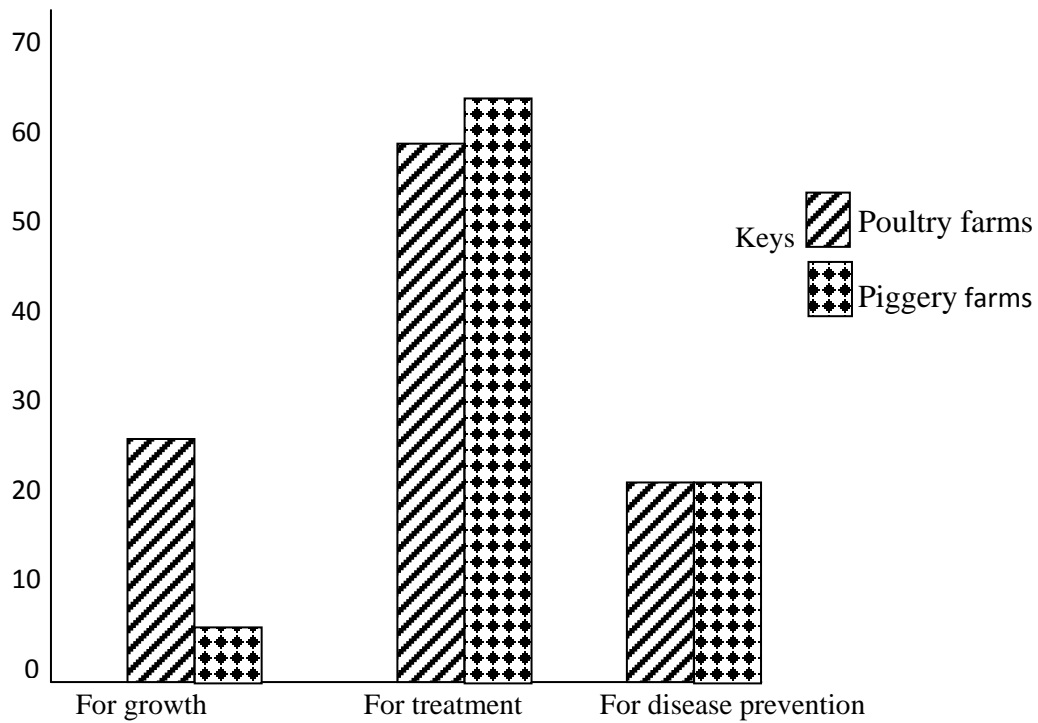


Figure 3: Reasons for the usage of antibiotics in poultry and piggery farms

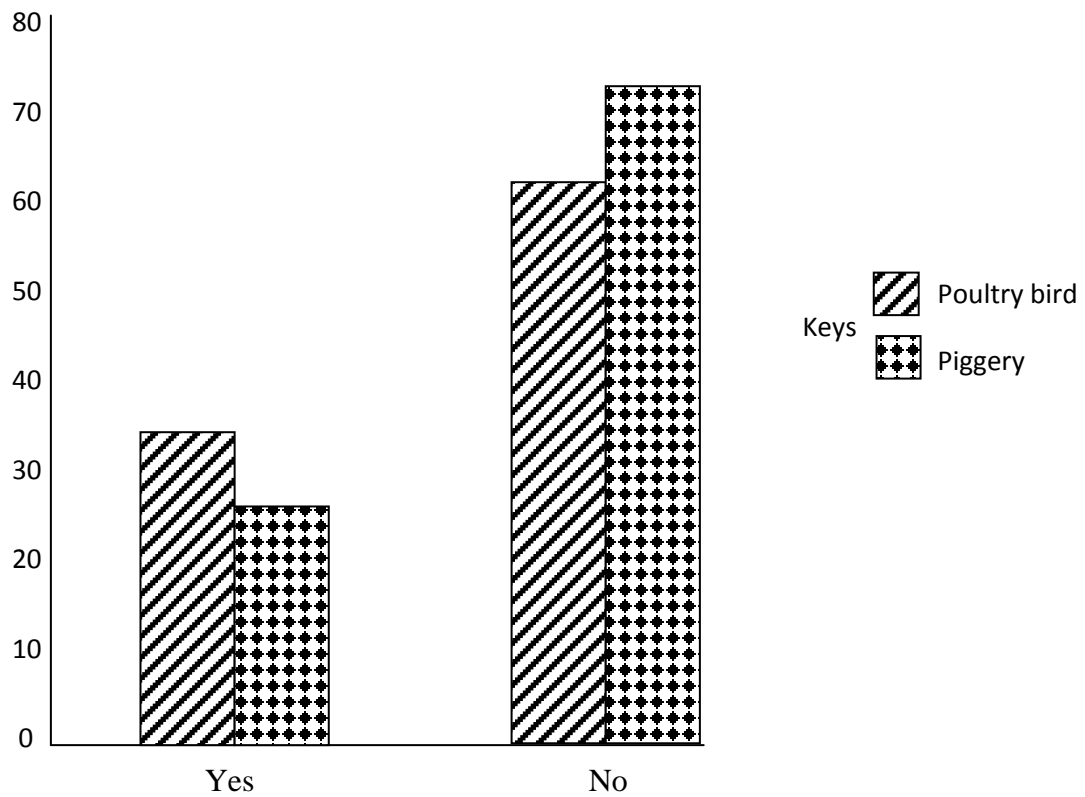


Figure 4: Laboratory analysis done prior to antibiotic use in poultry and piggery farms

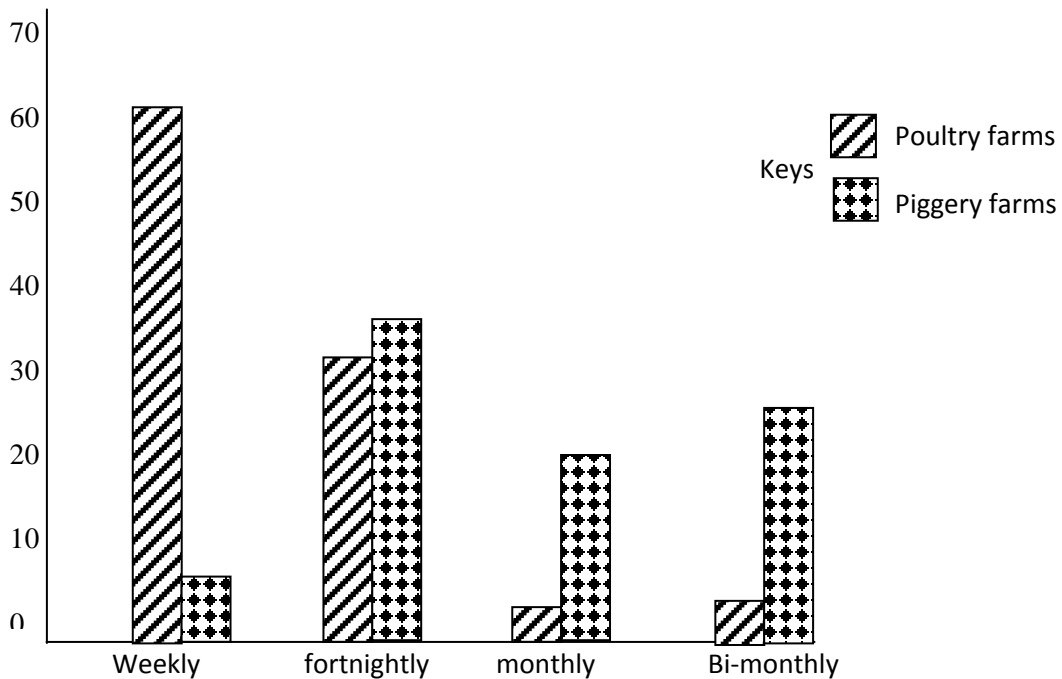


Figure 5 : Frequency of antibiotics use in poultry and piggery farms

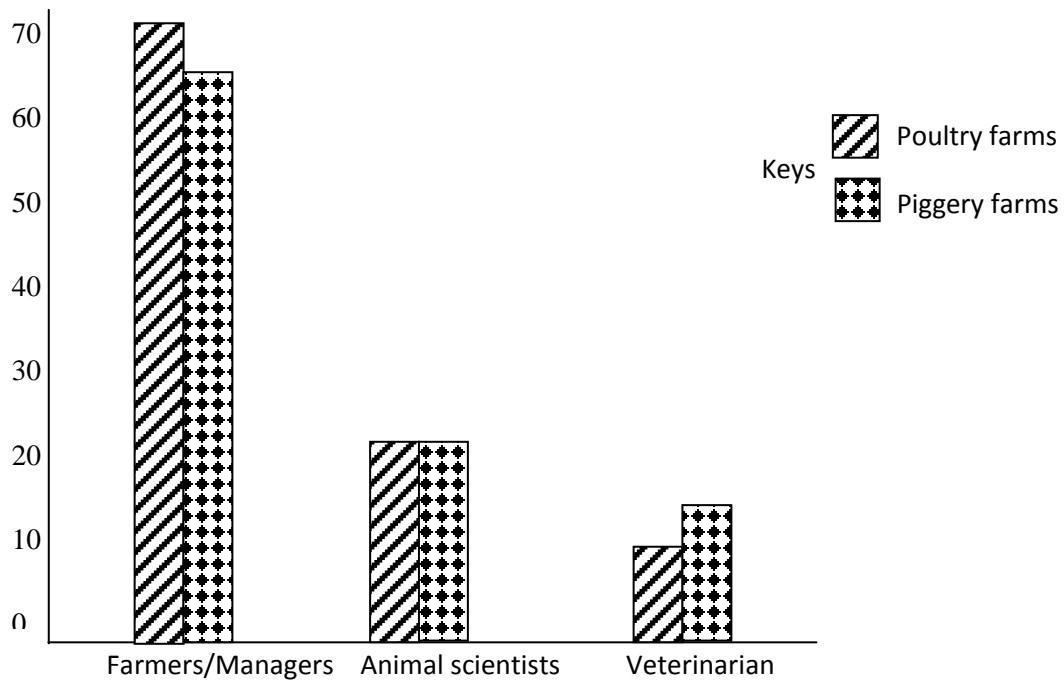


Figure 6 : Personnel that makes prescription of antibiotic usage

IV. DISCUSSION

Chlortetracycline (most commonly used at 400g/ton feed) and Streptomycin (30g/ton feed) were the two most effective and frequently use antibiotics for growth promotion, prophylactic and therapeutics and were in use in poultry birds and piggery farms respectively. Twenty-eight poultry farms reported regular sub therapeutic use of neomycin, while Streptomycin

and erythromycin were constantly used in one third of the poultry and piggery farms. If historical usage was also considered, tetracycline usage was most prevalent. In an earlier study, Sawant *et al*, (2005) reported that beta lactams and streptomycin were the most widely used antibiotics on livestock. Specifically, all the ten piggery and poultry farms that reported historical usage patterns has discontinued the use of tetracycline only 1 to 3 months prior to survey. Various other antibiotics

were in use at sub-therapeutic levels at some of the poultry and piggery farms, including a mixture of tetracycline, sulfathiazole, penicillin, lincomycin, virginiamycin, carbadox and tylosin. They were sometimes used simultaneously and sometimes sequentially during the production cycle, and the treatment varied in nature, dosage and duration. These results are consistent with results from a national survey performed by the National Animal Health Monitoring Survey (NAHMS) in which tetracycline, tylosin and bacitracin were the most commonly used antibiotics in grower and finisher animals (NAHMS, 2002). These results were also consistent with those of a slightly older survey of antibiotics use at Canadian swine farms in which tylosin was the most common antibiotic used in finisher animals (Dunlope *et al.*, 1998).

All poultry and piggery farms visited reported therapeutic usage of antibiotics in the last 4 years. The type of antibiotics used included tetracycline (all farms), penicillins and related cephalosporins (40% poultry farms, 30% piggery farms), the lincosamide Lincomycin (30% for both farms), the macrolides (50% for both farms), sulfa-drugs (60% for poultry farms and (40%) for piggery farms; and bacitracin (20% in both farm types). Therapeutic antibiotics were frequently used at high concentration through injection in pigs and through addition to water supply in poultry birds between 3-4 days duration. Sub-therapeutic antibiotics were routinely included at low levels in the feed of pigs and poultry birds. This may further add to the threat of antimicrobial resistance (Schjorring and Krogfeldt, 2011). Antibiotic prescriptions were mainly made by farmers/managers (70% in poultry, 65% in piggery farms). This is a contradiction on the use of chemical substances. Most of these prescriptions made without laboratory analysis done on samples for the isolation and typing of the pathogenic organism. The tendency to rely on personal experience for antibiotic use, dosage and withdrawal period could lead to improper antibiotics usage as reported by Zwald *et al.* (2004). Indiscriminate use of antibiotics may lead to the evolution of resistance by selecting directly for drug-resistant pathogens as well as for mobile genetic elements carrying resistance determinants to human and non-human animal pathogens. Antibiotics and antibiotic-resistant bacteria can be found in the air, ground water and soil around farms and on retail meat (Smith *et al.*, 2005), and people can be exposed to these pathogens through infected meat, vegetables fertilized with raw manure and water supplies contaminated by farm animal waste (Acar and Moulin, 2006).

Data on drug usage is essential for the development of national and international policies for containment of antibiotics resistance. This is seen in Denmark as reported by Stage *et al.* (2003) where data are collected at the farm level to include information

concerning animal species, age of animal, disease, and farm identification number, amount of medicine and date of use of medicine. Antibiotic usage data for the European countries was carried out by Grave *et al.* (2010). The rather large differences can be experienced by differences in types of animal production systems, different veterinary antibiotic policies and practices or differences in disease occurrence.

In conclusion, there was considerable variation in the management practices associated with antibiotic use on farms and reliable antibiotic use data for pigs and poultry birds are not publicly available, making it difficult to determine which drugs are used in what quantities and for what purposes. The absence of susceptibility testing and failure to consult veterinarian for treating animals, are some of the barriers to better surveillance. It can be inferred that antibiotics, particularly tetracycline and streptomycin, are extensively used for prevention and treatment of disease in pigs and poultry birds in Abia State.

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Drug Resistance Patterns of Bacterial Pathogens from Adult Patients with Pneumonia in Arba Minch Hospital, South Ethiopia

By Belayneh Regasa

Arba Minch University, Ethiopia

Abstract- Background: Community-acquired pneumonia (CAP) is associated with high mortality. Drug resistance is common in countries where the alternative treatments are limited and available drugs are misused. In resource limited countries; it is wise to determine antimicrobial susceptibility pattern of common bacterial pathogens of Community acquired pneumonia.

Methods: A cross sectional study conducted at Arba Minch Hospital, Southern Ethiopia from February to December 2013. Sputum specimens were collected; microbiological investigations and antimicrobial susceptibility testing were performed using standard procedures. Data was processed and analyzed with SPSS version 16.0.

Results: Out of 170 cases, only 73 (42.9%) were culture positive. Majority of tested bacterial isolates (>86%) were sensitive to Ceftriaxone and Ciprofloxacin. Most Streptococcus pneumoniae isolates (60%) were resistant to Oxacillin. Most of Staphylococcus aureus and gram negative bacterial isolates were resistance to Tetracycline (100%), Penicillin (83.3%), Ampicillin (50-100%), Doxycycline (50-100%), and Trimethoprim-sulfamethoxazole (83.3-100%). Multidrug resistance (MDR) was observed to most (60.3%) bacterial isolates.

Keywords: *pneumonia, bacterial pathogens and antimicrobial susceptibility pattern.*

GJMR-C Classification : *NLMC Code: WC 202*



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Conclusion: Antimicrobial resistance including Multidrug resistance was observed to a number of commonly used antibiotics, such as trimethoprim- sulfamethoxazole, penicillin groups and doxycycline. Hence, periodic monitoring of drug resistant pattern is essential for better management of community acquired pneumonia.

Keywords: pneumonia, bacterial pathogens and antimicrobial susceptibility pattern.

I. INTRODUCTION

Pneumonia affects 3–5 adults per thousand per year with a mortality of 7–14% in hospitalized patients (1). It is associated with high mortality. About 5.6 million cases of Community acquired pneumonia are reported in the United States each year, with an associated mortality rate of approximately 14% (2, 3). Despite the advent of potent antibiotic over the last decades, significant mortality is still associated with pneumonia (4). Increased antibiotic resistance in frequently isolated bacterial pathogens from pneumonia patients has complicated the selection process of

antimicrobial agents (5) and the clinical presentation is usually not specific enough to make a firm etiologic diagnosis (6). The resistant strains of bacteria can quickly multiply and spread within a community where antibiotic use is common. Consequently, antibiotic resistance often results in various societal costs, including increased drug costs, additional health-service costs (such as laboratory tests and hospitalizations), greater drug resistance-related morbidity and mortality, and productivity losses (7). So it is wise to determine antimicrobial susceptibility pattern of bacterial pathogens and this might help for the management of the case in case of emergency and helps for the rational utilization of antimicrobial agents.

II. METHODS AND MATERIALS

During the period February to December 2013 a total of 170 adults (above 15 years old) with typical symptoms of the disease, such as productive cough, fever, chest pain and the presence of consolidate on the chest radiograph consistent with pneumonia was included in this study. Sputum samples were inoculated onto Blood, MacConkey, Manitol Salt agar (MSA) and Chocolate agar (Oxoid Ltd, UK) plates (8). The bacterial isolates were then identified and subjected to antimicrobial susceptibility testing according to Clinical Laboratory Standards Institute (CLSI) recommendations (9, 10). The antibiotic discs used and their concentration were:- Ceftriaxone (CRO, 30µg), Ciprofloxacin (CIP, 5µg), Tetracycline (TE, 30µg), Chloramphenicol (C, 30µg), Erythromycin (E, 15µg), Doxycycline (DO, 30µg), Penicillin (P, 10µg), Gentamycin (CN, 10µg), Trimethoprim-sulfamethoxazole (TMP-SMX, 1.25+23.75µg), Ampicillin (AMP, 10µg) and Oxacillin (OXA, 1µg) All antibiotic were obtained from Oxoid Limited, Basingstoke Hampshire, UK. A standard inoculum adjusted to 0.5 McFarland was swabbed on to Muller- Hinton agar (Oxoid Ltd. Basingstoke Hampshire, UK); antibiotic disc were dispensed after drying the plate for 3-5 min and incubated at 37°C for 24 hours. For *S. pneumoniae*, MHA supplied with 5% sheep blood and for *H. influenzae*, MHA chocolate agar was used. Quality control strains that were used include: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC

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27853 (10). Selected Socio-demographic characteristics like age and sex were obtained. Data were entered and analyzed using SPSS version 16.0 computer software.

The proposal of this study was ethically approved by the Institutional Ethical Review Committee (IRC) of Arba Minch University. Permission was obtained from Medical director of Arba Minch Hospital. Written informed consent was obtained from each patient participated in the study.

III. RESULT

A total of 170 adult patients clinically diagnosed to have pneumonia in Arba Minch Hospital were selected and participated in this study (Table 1). Of these, 95 (55.9%) were males and 75 (44.1%) were females.

Table 1 : Distribution of pneumonia by sex and age (n=170)

Variables		Number (%)
Sex	Male	95 (55.9)
	Female	75 (44.1)
Age	15-25	23 (13.5)
	26-45	67 (39.5)
	46-65	63 (37)
	>65	17(10)

The isolated bacteria were, *Streptococcus pneumoniae* 20 (11.8%), *Staphylococcus aureus* 18 (10.6%), *Pseudomonas aeruginosa* 12 (7.1%), *Klebsiella pneumoniae* 11 (6.5%), *Escherichia coli* 5 (2.9%), *Proteus mirabilis* 2 (1.2%), *Proteus vulgaris* 1 (0.6%) and *Haemophilus influenzae* 4 (2.4%).

Streptococcus pneumoniae isolates showed relatively high resistance (60%) to Oxacillin (penicillin group representative) and all isolates were sensitive against Trimethoprim-sulfamethoxazole. High resistance rate *S. aureus* was observed to Tetracycline (100%), Oxacillin (83.3%), Ampicillin (83.3%), Penicillin (83.3%), Trimethoprim-sulfamethoxazole (83.3%), Erythromycin (50%) and Doxycycline (50%). *Pseudomonas aeruginosa* isolates showed that 50% resistant to Gentamycin. The antimicrobial testing of *K. pneumoniae* and *H. influenzae* isolates indicated that all isolates showed resistance (100%) to Tetracycline, Ampicillin and Trimethoprim-sulfamethoxazole. *Proteus* and *E. coli* isolates showed resistance to Tetracycline, Chloramphenicol, Doxycycline, Gentamycin, Ampicillin and Trimethoprim-sulfamethoxazole (Table 2).



Table 2 : Drug resistance pattern of bacterial isolates from adult patients with pneumonia in Arba Minch Hospital, 2013

Bacterial isolates	Drugs tested No(%) resistance											
	No	CRO	CIP	TE	C	E	DO	P	CN	TMP-STX	AMP	OXA
<i>S. pneumoniae</i>	20	NA	NA	10 (50)	1 (5)	1 (5)	NA	NA	NA	1 (5)	NA	12 (60)
<i>S. aureus</i>	18	4 (22.2)	4 (22.2)	18 (100)	5 (27.8)	9 (50)	9 (50)	15 (83.3)	5 (27.8)	15 (83.3)	15 (83.3)	15 (83.3)
<i>P. aeruginosa</i>	12	1 (8.3)	1 (8.3)	NA	NA	NA	NA	NA	7 (58.3)	NA	NA	NA
<i>K. pneumoniae</i>	11	0	0	11 (100)	2 (18.2)	NA	1 (9.1)	NA	2 (18.2)	11 (100)	11 (100)	NA
<i>P. mirabilis</i>	2	1 (50)	1 (50)	2 (100)	2 (100)	NA	2 (100)	NA	2 (100)	2 (100)	2 (100)	NA
<i>P. vulgaris</i>	1	0	0	1	1	NA	1	NA	1	1	1	NA
<i>E. coli</i>	5	0	0	5 (100)	5 (100)	NA	5 (100)	NA	1 (20)	5 (100)	5 (100)	NA
<i>H. influenzae</i>	4	1 (25)	1 (25)	4 (100)	1 (25)	NA	NA	NA	NA	4 (100)	2 (50)	NA
Total	73	7 (13.2)	7 (13.2)	51(83.6)	18 (29.5)	10 (26.3)	18 (48.6)	15 (83.3)	18 (36.7)	39 (63.9)	36 (87.8)	27 (71.1)

Note: NA- Note applicable, CRO- Ceftriaxone, CIP- Ciprofloxacin, TE- Tetracycline, C- Chloramphenicol, E- Erythromycin, DO- Doxycycline, P- Penicillin, CN- Gentamycin, TMP-STX- Trimethoprim-sulfamethoxazole, AMP- Ampicillin and OXA- Oxacillin

Multidrug resistance was also observed to a number of antimicrobial agents (Table 3)

Table 3 : Multi-drug resistance Antibigram of bacterial isolates from adult patients with pneumonia in Arba Minch Hospital, 2013

Bacterial Isolates	Resistance Antibigram	No (%)
<i>S. pneumoniae</i> (n=20)	OXA, TE	2 (10)
	OXA, TE, C, E	1 (5)
<i>S. aureus</i> (n=18)	OXA, TE, P, AMP	2 (12.5)
	OXA, AMP, E, DO, TMP-STX	1 (6.3)
	P, TE, E, DO, TMP-STX	1 (6.3)
	OXA, AMP, TE, E, DO, TMP-STX	2 (12.5)
	OXA, AMP, P, TE, E, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, E, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, DO, C, E, CIP, TMP-STX	1 (6.3)
	OXA, AMP, P, TE, C, E, CN, CRO, TMP-STX	2 (12.5)
	OXA, AMP, P, TE, C, E, CN, CRO, CIP, TMP-STX	4 (25)
<i>P. aeruginosa</i> (n=12)	CN, CRO	2 (20)
	CN, CRO, CIP	2 (20)
<i>K. pneumoniae</i> (n=11)	AMP, TE, TMP-STX	5 (45.5)
	AMP, TE, CRO, TMP-STX	2 (18.9)
	AMP, TE, CN, TMP-STX	2 (18.9)
	AMP, TE, C, TMP-STX	1 (12.5)
	AMP, TE, CN, CRO, TMP-STX	1 (12.5)
<i>P. mirabilis</i> (n=2)	AMP, TE, DO, C, CN, TMP-STX	1 (50)
	AMP, TE, DO, C, CN, CRO, CIP, TMP-STX	1 (50)
<i>P. vulgaris</i> (n=1)	AMP, TE, DO, C, TMP-STX	1 (100)

<i>E. coli</i> (n=5)	AMP, TE, DO, C, TMP-STX	3 (60)
	AMP, TE, DO, C, CN, TMP-STX	2 (40)
<i>H. influenzae</i> (n=4)	AMP, TE, TMP-STX	1 (33.3)
	AMP, TE, C, CIP, TMP-STX	1 (33.3)
Total (n=73)		44 (60.3)

IV. DISCUSSION

The importance of knowing susceptibility patterns of bacterial isolates in patients with pneumonia has been identified as a key step towards limiting unnecessary antibacterial prescribing and treating patients effectively, which was the main purpose of this study.

S. pneumoniae, which was the commonest isolate in this study, showed 60% resistant to oxacillin which is representative to penicillin group. This finding is comparable to studies conducted in USA (53%) (11) and Iran (30-57%) (12). In this study, most tested *S. pneumoniae* isolates showed that 95% susceptible to trimethoprim-sulfamethoxazole, but studies conducted in Nigeria (100%) (13) and Kenya (54%) (14), showed high resistance rate of *S. pneumoniae* to trimethoprim-sulfamethoxazole. In addition, 95% of tested *S. pneumoniae* isolates were susceptible to chloramphenicol and erythromycin. These findings are comparable to a study conducted in Kenya (>97%) (14). The second most causative agent *S. aureus* showed 77.8% susceptible to ceftriaxone and ciprofloxacin, and 72.2% to gentamycin and chloramphenicol. This result is comparable to studies conducted in Ibadan, Nigeria (66.7% ciprofloxacin and 66.7% gentamycin) (13) and Benin City, Nigeria (66.7% ceftriaxone, 66.7% ciprofloxacin, and 66.7% chloramphenicol) (15). In addition 83.3% of tested *S. aureus* showed resistance to penicillin, ampicillin, oxacillin and trimethoprim-sulfamethoxazole; which is comparable to studies conducted China (88.7% resistance to penicillin) (16) and Nigeria (resistance rate of 66.7% for penicillin) (13), but lower than study conducted in Nigeria (100% for trimethoprim-sulfamethoxazole) (13).

Most of tested gram negative bacilli isolates were sensitive (90%) to ceftriaxone and ciprofloxacin. These findings are comparable to studies conducted in Benin City, Nigeria (66-100%) (15) and Ibadan, Nigeria (60-100%) (13). Majority of gram negative bacilli was resistance (100%) to tetracycline, chloramphenicol, doxycycline (except *K. Pneumoniae*, 90% susceptible), trimethoprim-sulfamethoxazole and ampicillin. Similar study conducted in Nigeria (60-100%) (15), supports these findings. The commonest causative agent among gram negative bacilli, *P. aeruginosa*, showed 58.3% resistance to gentamycin, which is comparable to study conducted in Nigeria (53.6%) (13). However, it showed low resistance (8.3%) to ceftriaxone and ciprofloxacin;

but study conducted in Nigeria (39.3% resistance for ciprofloxacin) (13), showed high resistance. *K. pneumoniae* and *E. coli* showed 100% resistance to tetracycline, ampicillin and trimethoprim-sulfamethoxazole. These findings are comparable to studies conducted in Benin City, Nigeria (100% resistance to tetracycline) (15) and Ibadan, Nigeria (100% resistance to trimethoprim-sulfamethoxazole) (13). All tested *Proteus* species isolates were resistance (100%) to doxycycline, tetracycline, ampicillin and trimethoprim-sulfamethoxazole. These findings are comparable to study conducted in Ibadan, Nigeria (100% resistance to trimethoprim-sulfamethoxazole) (13).

All *H. influenzae* isolates tested for antimicrobial sensitivity showed low resistance (25%) to ceftriaxone, ciprofloxacin and chloramphenicol. These findings are comparable to study conducted in Nigeria (chloramphenicol 30.3% and ciprofloxacin 26.1%) (13). In most of tested *H. influenzae* isolates, high resistance rate to tetracycline (100%), ampicillin (50%) and trimethoprim-sulfamethoxazole (100%) were observed. These findings are similar with studies conducted in USA (47% resistance to ampicillin) (17) and Nigeria (93.7% resistance to trimethoprim-sulfamethoxazole) (13), but is not as high as that observed in other countries such as in China (>90% susceptibility to most antibiotics) (16).

The differences in antibiotic resistance patterns may be due to variations in the antibiotic prescribing habits in different geographical regions.

V. CONCLUSION

In the present study, most bacterial isolates were susceptible to ceftriaxone and ciprofloxacin. However, antimicrobial resistance including Multidrug resistance was observed to a number of commonly used antibiotics, such as trimethoprim-sulfamethoxazole, penicillin group and doxycycline. Hence, it is important to periodically monitor the antibiotic resistance patterns to aid physicians to choose empirical treatments for better management of pneumonia.

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Competing interest

The author declared that there is no any relevant competing interest to disclose in this research.

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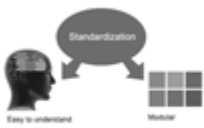
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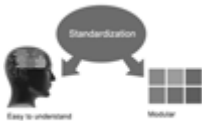
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1. General,
2. Ethical Guidelines,
3. Submission of Manuscripts,
4. Manuscript's Category,
5. Structure and Format of Manuscript,
6. After Acceptance.

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- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently. You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.



Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all, take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- 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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<i>References</i>	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring



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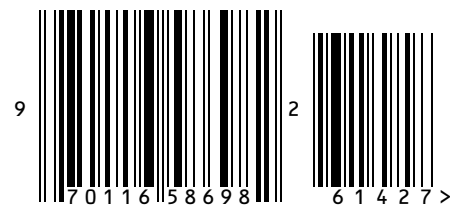
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