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Detection of Bla_{TEM} Resistance Gene in Bacteria Described as Nosocomial

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Abstract- During the year 1928, Alexander Fleming discovers the penicillin, substance capable of avoiding bacterial growth. Nevertheless, a little time later, bacteria capable of resisting penicillin and new antimicrobials. The above mentioned, still provokes concern in the scientific community, especially in the area of the Public Health, because the indiscriminate use of antimicrobial, subduing, mistakes in the administration frequency and the consumption of small amounts of antimicrobials from food produced by supply animals, are some of the factors that have influenced the appearance of resistant strains, due to the increase in selection pressure on these bacterial populations.

The bacterial aptitude to resist the action of antimicrobials is determined by genes present in the genome of the pathogen, which can be constitutive or skillfully incorporated using different mechanisms.

Considering the described precedents, in this work, by conventional PCR a fragment of the bla_{TEM} gene was detected in three resistant bacteria strains and sequenced, and it was high values of nucleotide identity respect to sequences of GenBank's database, which allowed to obtain three veterinary positive native controls for further investigations.

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I. Introduction

Nosocomial Infections. Nosocomial infections correspond to those infections acquired within a hospital ward and whose manifestation, depending on the incubation period, can occur 48-72 hours later, or even once the patient has obtained medical discharge (WHO, 2003). The illnes severity depends on how committed the patient is and the antimicrobial resistance capacity of the causative bacteria.

Nosocomial infections constitute an great risk of hospitalization and cannot be prevented always (Weese, 2010, Gaschen, 2008), presenting more frequently in intensive care units. This happens for several reasons: 1) the duration of the stay is usually longer than in other sectors of hospitals; 2) invasive medical processes such as the introduction of urinary catheters, allow the entry of opportunistic organisms; 3) the patients present immunocompromise or strong stress due to the disease they present or to the actual treatment and 4) the excessive use of antimicrobials allows the constant selection of resistant bacteria (Steele, 2009).

Public Health. The real impact of nosocomial infections in veterinary hospitals is not known, since the existing information, although, has constantly been increasing, remains very restricted to a small number of outbreaks and cases observed in reference hospitals (Weese, 2010). In human hospitals the incidence of nosocomial infections is lower than that observed due to the fact that patients spend less time in the Intensive Care Unit in relation to the human being, there is a lower prevalence of immunosuppressive diseases and there is a lack of infrastructure that allows a stay from the patients (Gaschen, 2010).

The main problems associated with nosocomial infections in dogs and cats are the transmission of multi resistant bacteria from patient to patient by the hands of hospital workers, transmission of infectious diseases from patient to patient and of zoonoses that could affect workers and even the owners of the patients (Gaschen, 2010).

Bacterial Nosocomiales Agents: Multiresistant bacteria commonly isolated in veterinary hospitals of small animals *include E. coli*, *Enterobacter* spp., *Enterococcus* spp., as well as several species of *Staphylococcus* and *Clostridium* spp. In Europe, *Acinetobacter baumannii* identified as resistant and causing nosocomial infections both in units dedicated to the care of small animals and in equine in a veterinary teaching hospital (Gaschen, 2010). Three of these bacteria have generated increasing concern as Vancomycin-resistant Enterococci (VRE), multiresistant E. coli and methicillin-resistant Staphylococcus aureus (MRSA) have appeared. The latter has been a problem for many years for human medicine, but today it generates great concern in the veterinary medical community, while multiresistant E. coli and MRSA are associated with nosocomial infections in hospitals of small and older animals, VRE are scarcely described as a nosocomial infectious agent in small animals (Steele, 2009).

Antimicrobial Resistance. Antimicrobial resistance is understood as the mechanism by which bacteria can reduce the action of antimicrobial agents (Fernández *et al.,* 2003). When the concentrations that the antimicrobial can reach in the organism do not exceed the minimum inhibitory substantially and, during prolonged times, although linked to the type of agent in question, the bacterium has all the possibilities to

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survive and is defined as resistant. In contrast, when the opposite occurs, the bacterium is defined as susceptible (Errecalde, 2004). This resistance can be a characteristic of an organism (intrinsic) or acquired by mutation or incorporation of genetic material (extrachromosomal DNA) or transposons (chromosomal or integrated to plasmids). The most frequent mechanisms of gene exchange between bacteria are conjugation, transduction, and transformation. The variations of these processes are developed through genetic engineering. An example of this is electrotransformation (Danchin *et al.,* 2002; Cabrera *et al.,* 2007). Depending on the antibiotic and the bacterial species, existe four types of resistance mechanisms: a) enzymatic inactivation of the antibiotic, b) modification of the site white; c) changes in the permeability of the bacterial membrane due to the substitution of outer membrane proteins (porins) when modifying its internal caliber; and d) expulsion of the antibiotic due to the overproduction of efflux pumps that prevents access of the antibiotic to the target site in the bacteria. The first mechanism described is one of the most common biochemical processes that provide resistance (Danchin *et al.,* 2002; Garza et al., 2009).

The Β-Lactams. Under this denomination, a continually growing number of antimicrobials is grouped, whose origin goes back to 1928 when Alexander Fleming discovered a substance capable of inhibiting the growth of *Staphylococcus aureus*. The β-lactams form the group of antimicrobials currently used in clinical practice and include penicillins, cephalosporins, carbapenems and beta-lactamase inhibitors, among others (Mediavilla and García-Lobo, 2004.). Structurally they have in common the β-lactam ring, associated with a thiazolidine ring, forming the 6-amino penicillanic acid responsible for the biological activity and a side chain changes that gives the antimicrobial and pharmacokinetic characteristics to the different antimicrobial derivatives (Mediavilla and García-Lobo, 2004). These chemical modifications reducing the capacity of antimicrobial resistance. The β-lactams inhibit the synthesis of the bacterial wall, acting in the final stage of peptidoglycan formation, called transpeptidation. This group of antimicrobials presents an analogy with an amino acid precursor of the chain of peptidoglycan, D-alanyl-D-alanine. Therefore they are capable of competing for the active site of an enzyme called "Penicillin-binding Protein" (PLP), which participates in the cross-linking of peptidoglycan. In this way the β-lactams are covalently bound to the active site of the PLP, which produces the irreversible inactivation of the enzyme, stopping the peptidoglycan formation and producing the osmotic lysis of the bacterial cell (Mediavilla and García-Lobo, 2004.). The introduction of a methoxyphenyl group to the side chain of the penicillins gave rise to methicillin, which is resistant to the enzymatic inactivation produced by the beta-lactamases of *Staphylococcus aureus*. By adding an amino group to the side chain of the Benzylpenicillin, the Aminopenicillins were created to broaden the spectrum of action of penicillins. Thus, ampicillin is effective against Gram-negative bacteria such as *Escherichia coli* and *Haemophilus influenzae* (Mediavilla and García-Lobo, 2004).

Bacterial Resistance To Β-Lactams. The most important mechanism of β-lactam resistance is the production of β-lactamases, which correspond to enzymes that hydrolyze the β-lactam ring, thereby becoming biologically inactive compounds. These enzymes are synthesized by Gram-positive and Gram-negative bacteria (Mediavilla and García-Lobo, 2004) and at present hundreds of β-lactamases are known, there are four types of them: 1) class A, represented by TEM-type β-lactamases; 2) class B, which correspond to rare metalloenzymes; 3) class C, represented by cephalosporinases of enterobacteria; 4) class D, represented by cloxacillinases.

The β-lactamases are encoded by the blaTEM gene. The term TEM is coined thanks to Temoniera, a patient from whom the first bacterium with characteristics of β-lactam resistance was isolated (Mediavilla and García-Lobo, 2004).

Molecular Genetics Techniques. The study and monitoring of these antimicrobial resistance genes have achieved thanks to the implementation of molecular genetic techniques, which allow detecting areas of interest in the genome of a microorganism.

The polymerase chain reaction (PCR) is one of these molecular methods, which consists in the in vitro synthesis of a white DNA sequence in a repetitive manner, thanks to the use of primers or primers (highly specific oligonucleotide sequence) They recognize small sequences that flank the segment of the genome to be amplified. This are achieved using Taq polymerase, an enzyme from the bacterium *Thermophilus aquaticus* that can incorporate free nucleotides at the 3'-end of the splitter, generating copies of the white sequence exponentially, at short intervals of time and high temperatures. It is characterized by its thermostability, high processivity (adds many nucleotides before being discarded) and high fidelity (Mullis and Faloona, 1987).

The PCR technique is consist in the consecutive repetition of three steps, which together make up a cycle. First, the target DNA is denatured, forming simple strands. This process is carried out at temperatures that vary between 90 and 96 ° C. The second step is the hybridization or annealing of primers to simple strands. The temperature of this stage is specific to each pair of primers. The third and final stage is the synthesis of DNA thanks to the action of a thermostable polymerase. The result is two double strands of DNA, formed by the original strand, and the new strand formed, which will be the target strand or template of the next cycle. This

process is repeat between 20 and 40 times (Mullis and Faloona, 1987). At the end of the series of cycles millions of copies of DNA zone will be generate, which are visualized as bands when performing electrophoresis, in which the DNA is separate according to its molecular weight and its negative electric charge. The DNA molecules are deposite in a polyacrylamide or agarose gel immersed in a buffer solution, which is subject to an electric field. The concentration of the gel determines the density of the solution, and therefore, the speed with which the molecules of interest move. Indeed, the smaller the size of the products of the reaction, the more concentrated the gel must be use; thus, diagnostic PCR generally uses 2% agarose concentrations (Mullis and Faloona, 1987; Danchin and Yuen, 2002; Pennington, 2002.). Thus, the objective of this report was to detect the bla_{TEM} gene involved in ampicillin resistance in bacteria described as nosocomial, through the polymerase chain reaction (PCR), subsequently from the obtained sequences; it was established the percentage of nucleotide identity concerning to official GenBank ® data. This will make an approximation of the situation presented by these genes in the hospital facilities of the University of Chile and a first approach of the subject in veterinary medicine within the country, to develop epidemiological studies to establish the behavior of this gene in the future.

II. Material and Methods

Samples. Three bacterial strains resistant to Ampicillin were studied, according to the Kirby-Bauer plate diffusion method, obtained in previous work (Jara *et al.,* 2009): *Pantoea agglomerans*, *Enterococcus faecium* and *Staphylococcus intermedius* (Table 1).

Table 1: Bacterial species suspected of carrying the blaTEM gene. According to Gram stain and antimicrobial susceptibility to Ampicillin

	Bacterias Gram (-)			Bacterias Gram (+)	
#	Especie	A	#	Especie	A
360	*Pantoea agglomerans*	R	034	*Enterococcus faecium*	R
A: Ampicilina: R: resistente.			P4	*Staphylococcus intermedius*	R

Bacterial DNA obtaining. The extraction of bacterial DNA was carried out using a commercial kit for extraction and purification (Genomic DNA Purification kit, Fermentas®), from cultures of 106 CFU / ml. Briefly, to 200 µL of bacterial culture, 400 µL of lysis solution was added, incubated for five minutes at 65 ° C, homogenizing manually every 1.5 minutes. Immediately, 600 µL of chloroform was added by gently mixing and inverting five times. Then it was centrifuged at 10,000 rpm for two minutes (Heraus Sepatech Biofuge®). After centrifugation, the upper phase is collected in an Eppendorf tube, and 800 µL of precipitation solution was added, mixed gently and centrifuged at 10,000 rpm for two minutes. The obtained pellet is resuspended by the addition of 100 µL of 1.2 M sodium chloride solution. To this mixture, 300 µL of cold ethanol is added and kept at -20 ° C for ten minutes. Then, it was centrifuged at 10,000 rpm for four minutes; the supernatant are removed and resuspended in 100 µL of nuclease-free water (Winkler®). Finally, this DNA was used immediately to perform the PCR test.

Detection of the blaTEM gene using the PCR technique. A 96-well Apollo thermocycler (CLP, USA) was used to carry out the polymerase chain reaction and a protocol that included the temperatures, the estimated time for each stage and the number of applicable cycles (Tenover *et al.,* 1994).

Partidores. The primers were used: 5'-TGGGTGCACGAGTGGGTTAC -3 'and 5'- TTATCC GCCTCCATCCAGTC -3' (Tenover *et al.,* 1994).

Mix the reaction to perform the PCR test. A 2X PCR Master Mix kit (Fermentas®), containing the thermostable polymerase, the deoxynucleotide triphosphates (dNTPs), the reaction buffer and MgCl2, was used. In a 0.2 mL Eppendorf tube, 15 µL of Master Mix, five µL of each of the primers and five µL of the DNA sample were added, obtaining a total volume of 30 µL. DNA amplification. The DNA amplification contemplates an initial denaturation at 94°C for one minute and then 30 cycles (94°C for two minutes, 57°C for one minute and 72°C for two minutes). Finally, a final extension are made at 72°C for ten minutes. The expected band size was 526 base pairs (Tenover *et al.,* 1994).

Visualization of the amplified products. It was performed by electrophoresis in 2% agarose gel (Winkler®) in TAE buffer (Fermentas ®). The PCR product was mixed with one µL of the commercial loading product, 6X Mass Ruler Loading Dye Solution (Fermentas ®), which it has glycerol to give density to the sample and bromophenol blue to verify the progress of the migration of the DNA bands. An aliquot of 6 µL of this mixture are deposit in the respective well of the gel.

Electrophoresis was carried out at 89 V for ninety minutes. As a molecular size marker, a standard containing DNA fragments between 100 and 2000 bp (DNA ladder (Fermentas ®)) was used. The gel was immersed in ethidium bromide (0.5 µg/mL) (Fermelo ®), and visualized in a transilluminator of ultraviolet light (Transilluminator UVP ®) to be finally photographed.

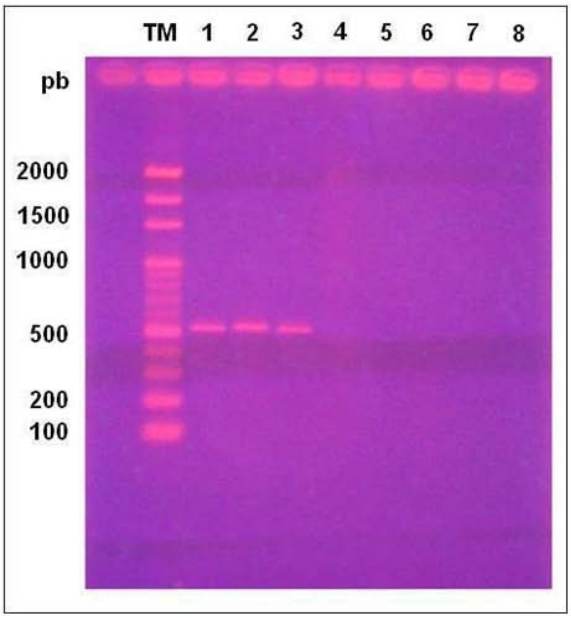
Biosafety measures. The laboratory work was carried out according to the biosafety levels established for the microbiology and animal virology laboratories, such as the use of clean material, correct waste disposal and the use of a closed apron and gloves in practical work. The process of visualization of the amplified product involves the use of ethidium bromide and a transilluminator of UV light, so when the gel was visualized an acrylic plate and glasses with UV filter were used. Subsequently, the elimination of the gel contemplated its incineration since ethidium bromide has -among others- mutagenic properties.

Sequencing. The DNA fragments were sent to the Sequencing Center of the GENYTEC company according to their requirements.

Analysis. From the sequences delivered by GENYTEC, a consensus sequence are obtained for each bacterium studied. Subsequently, the open-access online program called Clustal Ω was used to align the consensus sequences obtained together with the variants of the blaTEM gene fragment (GenBank accession number AF188199.1, and FJ668751.1 corresponding to *E. coli*; GU734697.1, GU734696.1, and GU734695.1 obtained from strains of *K. pneumoniae*;) and thus the percentage of nucleotide identity could be established (Thompson *et al.,* 1994,Genbank, 2018). Also, a multiple alignments was made between all the variants mentioned for the blaTEM gene and thus observe the percentage of nucleotide identity between them.

III. Results

Detection of the blaTEM gene in bacteria described as nosocomial. When carrying out the PCR according to the protocol established (Steele, 2009) in genetic material extracted from bacteria described as nosocomial, the expected white sequence are obtained. The bands obtained presented a molecular size of around 500 base pairs, and it was possible to visualize them clearly (Figure 1).



Lane	Content
TM	*Hyperladder II*. 100-2000 pb
1	DNA *P. agglomerans*
2	DNA *E. faecium*
3	DNA *S. intermedius*
4	DNA Feline herpes virus
5	DNA Canine herpes virus
6	cDNA Canine distemper virus
7	H_2O
8	Empty

Figure 1: Detection of blaTEM gene in three bacterial strains described as nosocomial

Sequencing and determination of nucleotide identity percentage (NIP) The sequences of the three amplified samples (in duplicate) were obtained satisfactorily (Annex 1). Multiple alignments using the Clustal Ω program of each consensus sequence and the official data for the bla$_{TEM}$ gene (GenBank accession number AF188199.1) indicated a high percentage of identity (≥93%) (Table 2). Also, it was observed that all variants of the blaTEM gene compared in this study, showed PIN close to 100%, which allows to assert that we are facing a highly conserved gene and therefore we can work with any of them in future studies.

Table 2: Nucleotidic Identity Percentage (NIP)

Percent Identity Matrix - created by Clustal2.1

1: S.intermedius	100.00	89.02	91.40	86.52	86.52	86.30	86.52	86.52	86.52	86.52	92.56
2: E.faecium	89.02	100.00	90.02	90.93	90.93	90.47	90.47	90.47	90.47	90.47	90.47
3: P.agglomerans	91.40	90.02	100.00	96.53	96.53	96.30	96.53	96.53	96.53	96.53	96.53
4: AY359287.1	86.52	90.93	96.53	100.00	99.90	97.89	99.53	99.52	99.53	99.42	99.54
5: AF427133.1	86.52	90.93	96.53	99.90	100.00	97.99	99.53	99.52	99.53	99.53	99.54
6: AF093512.1	86.30	90.47	96.30	97.89	97.99	100.00	99.84	99.84	99.84	99.65	99.77
7: GU734696.1	86.52	90.47	96.53	99.53	99.53	99.84	100.00	100.00	100.00	100.00	100.00
8: GU734697.1	86.52	90.47	96.53	99.52	99.52	99.84	100.00	100.00	100.00	100.00	100.00
9: GU734695.1	86.52	90.47	96.53	99.53	99.53	99.84	100.00	100.00	100.00	100.00	100.00
10: FJ668751.1	86.52	90.47	96.53	99.42	99.53	99.65	100.00	100.00	100.00	100.00	100.00
11: AF188199.1	92.56	90.47	96.53	99.54	99.54	99.77	100.00	100.00	100.00	100.00	100.00

IV. Discussion

Recognizing that antimicrobial resistance constitutes a threat to public health, since resistant agents increase the morbidity and mortality rates, increasing the patient's stay in a care center, the determination of resistance genes could guide the use of different therapeutic alternatives (Deutscher and Friedman, 2010). In this aspect, the results obtained in this study allow us to make some reflections on the resistance observed by the three bacterial strains against the beta-lactam used: First, the methods used: conventional PCR, electrophoresis in 2% agarose gel and visualization by ultraviolet light, allow obtaining a DNA fragment of around 500 bp, which is the first indication of the presence of the gene blaTEM, as previously mentioned in the literature (Arros, 2010; Tenover *et al.,* 1994). Thus, it will not be necessary to design other primers through any of the computer programs (*in silico* design) nor does it make necessary to make changes in the PCR protocol used (Tenover *et al.,* 1994). Secondly, by sequencing of the amplicons independently and obtaining the consensus sequence for each of them, the Clustal Ω program delivered valuable information both between the consensus sequences, as well as between each consensus sequence and the official data (GenBank: accession number AF188199.1): a PIN value greater than or equal to 93%. The latter is consistent with the fact that the blaTEM gene is a highly conserved gene in nature, reaching PIN values close to 100% when comparing various official GenBank data. This would make it possible to ensure that each amplicon - independent of origin - are a constitutive part of the blaTEM gene, each constituting in itself a native positive control for the detection of this gene in bacteria described as nosocomial, validated both by its size (~500 bp) as per its nucleotide sequence. Finally, in a future study it will be possible to have both a native control for nosocomial bacteria both Gram positive and Gram negative, which satisfies one of the primary objectives of this title memory. In the case of gram-positive environmental bacteria described as nosocomial, *Enterococcus faecium* (NIP = 96%) may be used and in the case of Gram-negative bacteria: *Pantoea agglomerans* (NIP = 96%).

V. Conclusions

In this title report, the bla$_{TEM}$ gene was detected from bacteria described as nosocomial, isolated from veterinary hospital facilities. This was demonstrated both by the presence of a DNA band of around 500 bp and by the nucleotide sequencing of the obtained amplicon. In addition, the obtained amplicons can be used as positive native controls of veterinary origin, for the first time in Chile.

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ANEXX 1: Sequences for Clustal Omega analysis

a) Consensus Sequences by Genytec

>*P.agglomerans* (consenso 1)

CCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGATCTGCTATCGGGCGCGGTATTATAACAGTATT
GACGCCAGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCAT
CTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACAACTGACAA
CGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGAG
CTGAATGAAGCTATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAAGGGCAACAACGTTGCGCAAA
CTATTAACTGGCGAACTACTTGC

>*E.faecium* (Consenso 2)
CCTTGAGAGTTTTCGCCCCGAAAAACGTTTTCATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTATTAACCAAATATTGA
CGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCT
TACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCATAACCATGAGTGATAACACTGCGGCCAACTTAAAAAAGACAACG
ATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCAGTAACTCGCCTTGATCGTTGGGAACCGGTTTCTG
AATGAAGCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAA
CTACTTAC

>*S.intermedius* (Consenso 3)
CCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCAATGATGAGCAGTTTTAAATTTCTGCTATCTGGCGCGGTATTATCGCGTATTGA
TTCTGGGCAAGAGCAACTCGGTCCCCGCATACACTATTCTCAGAATGACTTGCTTGAGTACTACCAGTCACAGAAAAGCATCTTA
CGGATCGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATCACACTGCGGCCAACTTACTTCTCACAACGAT
CGGAGGACCGAACGAGCTAACCGCTTTTTTGCACAACATGCGGGATCATGTAACTCGCCTTGATCGTTGGGAACCGGTTTCTGA
ATGAAGCCATACCAAACGCCGAGCCTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCTAACTATTAACTGGCGAA
CTACTTAC

b) Oficial sequences by Genbank

>GU734697.1 >GU734696.1 >GU734695.1 >FJ668751.1 >AF188199.1 >AY359287.1 >AF427133.1
>AF093512.1

ANEXX 2: Clustal Aligment

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S. intermedius      CGCCCCGAAGA-ACGTTTTCAATGATGAGCAGTTTTAAATTTCTGCTATCTGGCGCGGTA   72
E. faecium          CGCCCCGAAAAA--CGTTTTCATGATGAGCACTTTTAAAGTTCTGCTATGTGGCGCGGTA   71
P. agglomerans      CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGATCTGCTATCGGGCGCGGTA   73
AY359287.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
AF427133.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
AF093512.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   419
GU734696.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   176
GU734697.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   180
GU734695.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   180
FJ668751.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   233
AF188199.1          CGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTATGTGGTGCGGTA   73
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S. intermedius      TTATC-GCGTATTGATTCTGGGCAAGAGCAACTCGGTCCCCGCATACACTATTCTCAGAA   131
E. faecium          TTAACCAAATATTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   131
P. agglomerans      TTATAACAGTATTGACGCCAGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   133
AY359287.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
AF427133.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
AF093512.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   478
GU734696.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   235
GU734697.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   239
GU734695.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   239
FJ668751.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   292
AF188199.1          TTATC-CCGTGTTGACGCCGGGCAAGAGCAACTCGGTCGCCGCATACACTATTCTCAGAA   132
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S. intermedius      TGACTTGCTTGAGTACTA-CCAGTCACAGAAAAGCATCTTACGGATCGCATGACAGTAAG   190
E. faecium          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   191
P. agglomerans      TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   193
AY359287.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
AF427133.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
AF093512.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   538
GU734696.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   295
GU734697.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   299
GU734695.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   299
FJ668751.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   352
AF188199.1          TGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAG   192
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S. intermedius      AGAATTATGCAGTGCTGCCATAACCATGAGTGATCACACTGCGGCCAACTTACTTCTCAC   250
E. faecium          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTAAAAAAGAC   251
P. agglomerans      AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACAACTGAC   253
AY359287.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC   598
AF427133.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCGGCCAACTTACTTCTGAC   598
AF093512.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   598
GU734696.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   355
GU734697.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   359
GU734695.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   359
FJ668751.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   412
AF188199.1          AGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACTGCTGCCAACTTACTTCTGAC   252
                    ********************************** ******* *********    **

S. intermedius      AACGATCGGAGGACCGAACGAGCTAACCGCTTTTTTGCACAACATGCGGGATCATGTAAC   310
E. faecium          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCAGTAACT   311
P. agglomerans      AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   313
AY359287.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
AF427133.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
AF093512.1          AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC   658
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GU734696.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  415
GU734697.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  419
GU734695.1        AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  419
FJ668751.1       AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  472
AF188199.1       AACGATCGGAGGACCGAAGGAGCTAACCGCTTTTTTGCACAACATGGGGGATCATGTAAC  312
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S. intermedius    TCGCCTTGATCGTTGGGAACCGGTTTCTGAATGAAGCCATACCAAACGCCGAGCCTGACA  370
E. faecium       CGCCTTGATCGTTGG-GAACCGGTTTCTGAATGAAGCCATACCAAACGACGAGCGTGACA  370
P. agglomerans    TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCTATACCAAACGACGAGCGTGACA  372
AY359287.1       CCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
AF427133.1       CCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
AF093512.1       TCGCCTTGATAGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  717
GU734696.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  474
GU734697.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  478
GU734695.1        TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  478
FJ668751.1       TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  531
AF188199.1       TCGCCTTGATCGTTG-GGAACCGGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACA  371
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S. intermedius    CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCTAACTATTAACTGGCGAACTACTTA  430
E. faecium       CCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  430
P. agglomerans    CCACGATGCCTGTAGCAAGGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTG  432
AY359287.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  777
AF427133.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  777
AF093512.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  777
GU734696.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  534
GU734697.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  538
GU734695.1        CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  538
FJ668751.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  591
AF188199.1       CCACGATGCCTGCAGCAATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTA  431
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