Veterinary Sciences and Medicine

RESEARCH ARTICLE

Detection of Resistance Genes to Biocides in Bacteria Nosocomiales by Means the Chain Reaction of the Polymerase

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Abstract

Infections represent the greatest threat to animal and human health, for this reason since ancient times, ways to control them have been sought, and in the first years of the 20th century, the first chemo-therapeutic molecules (sulfas) and the first antibiotic were developed. (Penicillin), which were used clinically in the forties, managing to control infections that until then were extremely high morbidity and difficult to treat. Nosocomial infections are those acquired in a hospital and manifested 48 to 72 hours after the admission of patients. They are caused by ubiquitous, opportunistic and highly resistant bacteria, which preferentially affect those patients with varying degrees of immune compromise, affecting their evolution and recovery. Disinfectants and antiseptics (biocides) are used in medical practice as an essential part of the biosafety protocols adopted to minimize the spread of this type of infections among staff, patients and hospital facilities, but even these compounds have shown resistance.

These bacteria have multiresistance phenotypes (defined as resistance to two or more antimicrobials) that reflect the great influence of the hospital environment in the emergence of mechanisms of insensitivity to different toxic compounds, which are found in residual concentrations (sub-lethal) in the environment, either by the acquisition of mutations or, by the acquisition of gene cassettes, which, in turn, allow the selection of successful bacterial clones and the exchange of resistance genes included in integrons, plasmids and / or transposons. These, together with the efflux pumps, explain the intimate relationship between multiple resistances to antimicrobials and that observed to biocides.

Resistance to biocides represents one of the greatest threats to public health, because the mechanisms involved are characterized by being nonspecific, affecting a wide range of substances not structurally related. For this reason, efforts have been made to complement the epidemiological surveillance of antimicrobial resistance, investigating the resistance to biocides by means of molecular diagnostic techniques, within which the detection of the genes involved by means of polymerase chain reaction delivers relevant information in the development of new control strategies, vigorously adapting the protocols used to the epidemiological reality.

In response to this emerging need, this work has been focused on the detection of genes of resistance to biocides in nosocomial isolates, as a first approach in national veterinary medicine, to provide knowledge that allows the control of this type of infections. None of the most representative genes of the MFS families (qacA and qacB) and SMR (qacC and qacD) were detected in the 80 samples analyzed, obtained from clinical-veterinary units of the University, from both the Bilbao Veterinary Hospital and of the Faculty Hospital. The concentrations of biocides used in the clinical-veterinary units of the University were sufficient to inhibit the bacterial growth of samples taken directly from the sprinklers and hand brushes, but not in the rest of the sampled places.

Introduction

Bacteria are characterized by being ubiquitous in nature. As part of the normal flora of individuals, they also have a low pathogenicity and virulence, which allows them to live in harmony with their host. These bacteria are constantly transmitted between animals, without constituting a risk to health. However, in the hospital environment bacterial populations coexist under a highly variable and demanding environment, which exposes them to various stress situations, which as a selective pressure force; have been the main cause of the emergence of highly resistant bacterial strains. This is a problem because medical personnel, supplies and instruments are the most important transmission elements among patients [1-3]. In veterinary medical practice, this phenomenon also exists, and for methicillin-resistant Staphylococcus aureus (MRSA), other species of the genus (S. intermedius and S. pseudointermedius), and Escherichia coli have demonstrated the important role of personnel as carriers and potential source of disseminating this type of pathogen [4-7]O 'Mahony et al., (2005) [8-10].

The acquisition of multi-resistant pathogens as commensals is the first stage in the pathogenesis of nosocomial infection. Despite not having the molecular machinery of virulence (specialized toxins, transport systems type III, etc.), the acquisition of these

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Received: Oct 29, 2018; **Accepted:** Nov 03, 2018; **Published:** Nov 05, 2018

infections occurs mainly in immunocompromised individuals, under a regimen of antimicrobials or invasive procedures with the use of antiseptics by Long periods of time. This favors the multiplication of those infecting bacteria with the genetic-molecular potential of resisting them, being able to generate serious infections that compromise the survival and recovery of patients due to the nature of the infectious process and their non-response to treatment [11, 12].

In medicine, this topic has been under investigation for decades, and currently, the bacteria with the greatest clinico-epidemiological importance are methicillin-resistant S. aureus (MRSA) and vancomycin-resistant enterococci (ERV), both Gram-positive. Gram-negatives such as Pseudomonas aeruginosa and enterobacteria follow in importance [13, 14].

Biocide is all that chemical compound used to destroy life forms; However, this definition is quite general and some authors prefer to call germicide to everyone used to destroy or inhibit the growth of microorganisms. According to their effectiveness, they are called sterilizers (they destroy all vegetative forms and spores), disinfectants (they reduce the bacterial load, due to their greater toxicity they must be used on inert surfaces) and antiseptics (applied on living tissue). In the context of medical practice, the last two groups are the most frequently used, which is why they will be given special attention [15, 16]. Germicides at high concentrations produce a generalized damage to the bacterial ultra-structure, both at the cellular membrane level and in intracellular components, thus differentiating itself from the specific action of antimicrobials (which recognize specific target molecules). In addition, they are used at much higher concentrations, generally lethal for eukaryotic cells, while antimicrobials have a selective toxicity. Despite this, it has been described that low concentrations of triclosan act specifically by inhibiting the enzyme enoyl reductase, responsible for the synthesis of essential fatty acids, a mechanism that exerts a bacteriostatic effect [17-20]. The biocidal activity of a compound varies markedly between the different types of microorganisms and even between different strains of the same species. Among the vegetative forms, the most resistant are the mycobacteria, then the Gram negative ones, being more sensitive the Gram positive and mycoplasmas. This is partly due to differences in the composition of the cell wall [21]. Biocides vary widely in their chemical structures and although the precise mechanism of action reflects this characteristic, when they are used in high concentrations they show considerable similarity [22].

Bacterial resistance: a manifestation of the adaptive capacity of the procariontes.

The first organisms to be able to colonize, survive and create the optimal atmospheric conditions for the emergence of new life forms were bacteria. Since that ancestral procarionte have derived all current bacterial forms, which despite the various demands that represent the fluctuating environment in which they inhabit, have managed to adapt progressively, becoming the predominant life forms on the face of the earth [23, 24].

Since ancient times the human being has had to face the challenge of controlling and treating infections, but with the development of medicine and the discovery of antibiotics and synthetic chemotherapeutic agents (encompassed under the term antimicrobial) it was possible to increase life expectancy and decrease the recovery time of those infected patients. However, shortly after its discovery, the first enzymatic mechanism capable of inactivating penicillins was described [25]. In response to this latent threat began the exhaustive search for new therapeutic molecules, despite this, the phenomenon continues to increase to such an extent that every time you start using a new antimicrobial (as a treatment of highly resistant infections) its widespread use, Irrational and truly unjustified, it allows that shortly after resistant strains emerge from several bacterial species [26-28]. The latter highlights the need to use antimicrobials with a criterion rather than the simple medical experience and the theoretical framework that encompasses each therapeutic alternative, since currently novel molecules such as moxifloxacin, extended spectrum cephalosporins, carbapenemic derivatives and monobactams are ineffective when treating bacterial infections, especially nosocomial infections [13]. In fact, it has been shown that the use of cephalosporins, carbapenems and fluoroquinolones is a well-known risk factor for the acquisition of MRSA in humans, suggesting that the selective pressure generated by antibiotics has contributed to the prevalence of these infections [29-31].

Material and Methods

Samples

Gram-negative (n = 32) and Gram-positive (n = 48) bacteria considered as nosocomial (n = 80), obtained at the Veterinary Hospital of the University of Chile (offices Bilbao and Faculty) and previously characterized between 2007 (n=51) and 2008 (n = 29) that present a multiresistance profile to antimicrobials.

Obtaining bacterial DNA

From a bacterial culture of 106 CFU / mL, the genomic material was extracted by means of a commercial extraction and purification kit (Genomic DNA Purification Kit, Fermentas ®). Thus, to 200 µL of sample, 400 µL of lysis solution was added, incubated at 65°C for 5 minutes, homogenizing manually every one and a half minutes. Immediately afterwards, 600 µL of chloroform (Merk®) was added, gently mixing (inverting the tubes 5 times) and then centrifuging at 10,000 rpm for two minutes (Heraus Sepatech Biofuge®). Parallel to the centrifugation, the precipitation solution was prepared by adding 720 µL of nuclease-free water (Winkler®) to 80 µL of the precipitation concentrate (10X) provided by the kit. Once the centrifugation was finished, the upper phase was collected in a 1.5 mL Eppendorf tube and 800 µL of precipitation solution was added, gently mixing and centrifuging at 10,000 rpm for 2 minutes. The obtained pellet was dissolved in 100 µL of a 1.2 M NaCl solution provided by the kit. To this mixture was added 300 µL of cold ethanol and kept at -20°C for 10 minutes to precipitate the DNA. Then it was centrifuged at 10,000 rpm for 4 minutes, the ethanol was removed, and the

obtained pellet was dissolved in 100 µL of nuclease-free water. This DNA was used immediately to perform the PCR test or stored at 4°C for no more than one month.

PCR reaction mixture

To achieve the amplification mixture of the purified DNA, a 2X PCR Master Mix kit (Fermentas®) was used, which includes the thermostable polymerase, the deoxynucleotide triphosphates (dNTPs), the reaction buffer and $MgCl_2$, from which 12.5 µL were extracted. Were deposited in a 0.2 mL Eppendorf tube, together with 5 µL of each of the primers, and 5 µL of the hardened DNA sample, obtaining a total volume of 27.5 µL. We proceeded to its homogenization using the vortex to ensure the mixing of the reagents.

Partidores and PCR protocol

1. **Smr gene:** the sequence of primers was selected for its high specificity. Amplify segments of known size of 285 base pairs (bp), and correspond to: Smr-F: 5'-ATAAGTACTGAAGTTATT GGAAGT - 3 ' (24 bases). Smr-R: 5'- TTCCGAAAATGTTTAACGAAACTA - 3 ' (24 bases).
2. **qacA/qacB gene:** the sequence of primers was selected for its high specificity. Amplify segments of known size of 361 bp and correspond to: QacA/B. F: 5'- GCAGAAAGTGCAGAGTTCG - 3' (19 bases). QacA/B R: 5'-CCAGTCCAATCATGCCTG - 3' (18 bases).

The reaction protocols for each pair of primers are based on those standardized by [32 and 1].

Visualization of PCR products

It was performed by electrophoresis in 2% agarose gel (Winkler®) in Tris acetate EDTA (TAE) buffer (Fermentas ®), which was then subjected to electrophoresis, immersed in ethidium bromide (0.5 µg / mL). (Fermelo ®). The PCR product was mixed with 6 µL of the commercial loading product, 6X Mass Ruler Loading Dye Solution (Fermentas ®), which has glycerol to give density to the sample and bromophenol blue to check the progress of the migration of the bands of DNA An aliquot of 6 µL of this mixture was deposited in the respective well of the gel. Electrophoresis was carried out at 90 V for 90 minutes. 5 µL of Favorgen 100 bp DNA ladder (Favorgen ®) was used as molecular size marker, which contains DNA sequences between 100 and 3000 bp to facilitate the detection of the amplified fragments. Once finished, the gel already stained with ethidium bromide was visualized in the ultraviolet transilluminator (Transiluminator UVP ®), and photographed.

Results

Detection of genes that give resistance to biocides: When performing the conventional PCR technique on samples of DNA extracted from the 80 strains isolated and characterized as multiresistant to different antimicrobials according to the Kirby-Bauer sensitivity determination method, none were positive for the detection of the qacA, qacB genes, qacC and qacD (Figure 1).

Figure 1:2% agarose gel stainedwith ethidium bromide and visualized in UV transilluminator.Lanes 1 to 4: PCR productsfor gene qac A/B. Lane 5: MTM molecular size DNA Favorgen® 100 bp. Lanes 6 to 9: PCR productsforsmr gene. Lanes 1 and 6: isolate 083 (E. faecium). Lanes 2 and 7: 548M isolate (P. aeruginosa). Lanes 3 and 8: isolate MRSA (S. aureus, clinicalisolate). Lanes 4 and 9: 519M isolate (E. coli).

In Figure 1 -representative of 8 negative electrophoresis- no bands or fragments of DNA of a size around 285 base pairs (smr gene) are observed. Similarly, bands or fragments of DNA of a size compatible with the amplicon of the qacA / qacB gene (361 base pairs) are not appreciated.

Discussion

In the first place, the absence of the four genes in the 80 strains characterized is not an unexpected result, since these determinants of resistance are described more frequently in the species S. aureus, not being exclusive of it. There is a large variety of qac genes (qacA-qacJ) that are described in both Gram positive and negative. In the latter, the determinants with greater epidemiological relevance correspond to qacE and qacEΔ1, due to their association with integrons of class I. However, qac A/B and qacC/D have been described less frequently in Gram negative species, including to those belonging to the genera Pseudomonas (P. putida, P. syringae, P.mendocina), Escherichia, Enterobacter and Serratia, as well as Gram positive genera Enterococcus, Streptococcus, Clostridium and Bacillus, highlighting the important physiological role, different from resistance, that these pumps fulfill in nature.

The result of this work corroborates the scarce frequency of these genes in Enterobacteriaceae and P. aeruginosa, although this result is subject to a margin of error derived from the sampling process, in which those colonies that possessed any of the determinants, due to the immense size of the bacterial

population, both in the hospital and in the clonal expansion during laboratory culture. Second, it is important to consider that these genes are located in plasmids, extrachromosomal elements that have intrinsic characteristics of stability and heritability according to different environmental conditions, in this way, during growth under laboratory conditions and in the absence of selective pressure (which give advantage to the clones that own them) become a cost for the bacterial cell, determining that those cells that lack them have shorter generation times, and therefore, grow at a faster rate, displacing the carrier population . Some authors [1, 2] prior to the detection of resistance genes submitted isolates to sensitivity tests to biocides (which can act as a selective pressure, favoring carriers).

Thirdly, although during the experiments a positive control was not available to indicate the correct functioning of the reaction, it should be noted that the negative result is not due to faults in the DNA purification process, since the same purified was used to successfully amplify 7 other genes (blaTEM, mecA, tetO and tetM), including 3 genes of 35 efflux pumps of the MFS family that deliver resistance to tetracyclines (tetA, tetB and tetK) (Figure 2).

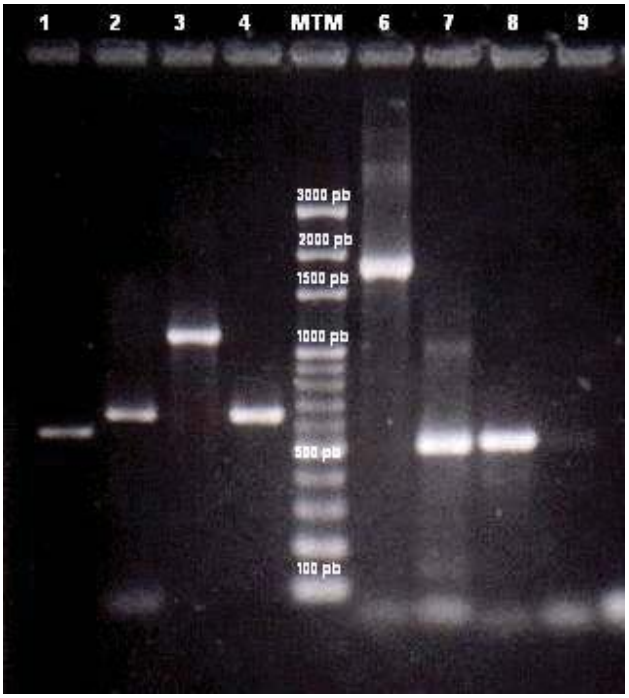

Figure 2:2% agarose gel stained with bromide ethidium and visualized in UV transilluminator.
The image shows other genes detected for the isolates of photograph 1 using the same purified DNA. Lanes 1 and 6: 083 (*E. faecium*) *tet*K genes and *tet*M, respectively. Lanes 2 and 7: 548 M (*P. aeruginosa*) *tet*B and bla_{TEM} genes. Lanes 3 and 8: MRSA (*S. aureus*, isolated clinical) *tet*K and *mec*A genes. Lanes 4 and 9: 519M (*E. coli*) *tet*B and bla_{TEM} genes. The molecular size marker of DNA Favorgen® 100 bp (MTM, lane 5) shows the approximate sizes of each band representative of the *tet*B genes (650 bp), *tet*K (600 bp and 1159 bp), *tet*M (1723 bp), bla_{TEM} (526 bp) and *mec*A (533 bp).

The primers have been used in other successful studies, and the only variability phenomenon to consider is the concentration of magnesium and the polymerase used in the reactions. However, the magnesium concentration of the protocol is the same as that included in the Reaction Master Mix (Annex 2). Additionally, a colored Master Mix whose composition includes another Taq polymerase was used, obtaining the same results (Mango Mix 50 mM solution of $MgCl_2$, Bioline).

Fourth, the importance of efflux pumps in medicine is that a bacterium can acquire multiple resistance in a single step, in a non-specific manner, similar to the acquisition of resistance plasmids. It is important to consider that, the ubiquity of these pumps between microorganisms and the irrational use of antibiotics and chemotherapeutics, will be the main factors responsible in promoting the increase of bacterial resistance in the medium and long term, not only to the drugs used, but also to others not related and unknown by bacteria, thanks to the low specificity of these protein systems in the recognition of their substrates [33]. For this, for future studies it would be necessary to search for these genes in clinical isolates of MRSA in order to obtain a positive control for the genes qac AD (described in the literature as its main vector) or, failing that, request clones of these genes to foreign researchers

Additionally, in order to increase the sensitivity of the entire procedure, it would be interesting to implement techniques for the determination of susceptibility to cationic biocides prior to detection by PCR, in order to exert a selective pressure to maintain the carrier plasmids in the population of samples. Consequently, it is necessary to continue this important study, with the search of conserved gene segments of the 5'-end integrase, together with the 3'-end genes, qacE and qacEΔ1 of class I integrons, in these same isolates and in new ones. In addition, of the conserved genetic sequences of the 5'- and 3'-endings of class II integrons, and of the transposase / integrase of class III conjugative transposons with the aim of establishing structural and functional relationships between these mobile elements, the genes of resistance to biocides and antimicrobials studied by the project, and the phenotypes observed.

For the detection of class I integrase [34]standardized a protocol using the intI-F primers: CCTCCCGCACGATGATC and intI-R: TCCACGCATCGTCAGGC, while [35] for the amplification of the variable region of the class integrons I, standardized a protocol using the 5'CS-F primers: GGCATCCAAGCAGCAAG and 3'CS-R: AAG CAGACTTGACCTGA.

These allow to amplify and sequence the variable region to characterize the circulating gene cassettes (included in them), and their impact on the resistance observed in a given bacterial population. However, these authors consider the sul 1 gene as conserved motif of the 3'-end, so the reaction leaves out those integrons of class I that replace it with a determinant qacE, since the former possess the variety qacEΔ1, originated from the interruption of the sul1 gene in the qacE sequence.

There is some ignorance about the association between genes of resistance to biocides with integrons of class II and conjugative transposons, mobile genetic elements of great impact in the phenomenon of antimicrobial resistance, because they transport a great variety of genes involved in delivering resistance to many compounds [36]. The latest generation of therapeutics, while integrating not only the chromosome, but also plasmids, being the transferable elements par excellence and one of the main threats to public health.

Goldstein [34] standardized the reaction using primers to detect integrase proper to integrons of class II (intI2-F: TTATTGCTGGGATTAGC and intI2-R: ACGGCTACCCTCTG TTATC), while L'Abée- Lund and Sorum (2001) standardized a reaction to detect and characterize their variable region, using primers 5'CS-F: GACGGCATGCACGATTTGTA and 3'CS-R: GATG CCATCGCAAGTACGAG that recognize sequences conserved from the extremes and initiate the amplification of the variable internal region.

Finally, and as a projection of this study, using Vector NTI Advance™ 9.1 software from Invitrogen ™ an alignment of nucleotide sequences (obtained from Genbank) was performed to identify the most conserved regions between the variants qacE and EΔ1 (from different bacterial species, in order to design a pair of primers (using the Primer3™ version 4.0 and Netprimer ™ programs from PREMIER Biosoft International) that recognizes common conserved sequences, thus allowing to amplify the biocide resistance determinant qacE / qacEΔ1, and additionally, the variable region of a larger population of integrons, when considering the previously mentioned source of variation, using them together with the primers for the integrase described by [34].

References

1. Noguchi N, Suwa J, Narui K, Sasatsu M, Ito T, et al.(2005) Susceptibilities to antiseptic agents and distribution of antiseptic-resistance genes qacA/B and smr of methicillin-resistant Staphylococcus aureus isolated in Asia during 1998 and 1999. *J Med Microbiol* 54: 557–565. [View Article]
2. Vali L, Davies S, Lai L, Dave J, Amyes S(2008) Frequency of biocide resistance genes, antibiotic resistance and the effect of chlorhexidine exposure on clinical methicillin-resistant Staphylococcus aureus isolates. *J Antimicrob Chemother* 61: 524-532. [View Article]
3. CDC: Centers for Disease Control and Prevention(2018)Standard Precautions.[View Article]
4. Seguin JC, Walker RD, Caron JP, Kloos WE, George CG, et al. (1999) Methicillin-resistant Staphylococcus aureus outbreak in a veterinary teaching hospital: potential human-to-animal transmission. *J Clin Microbiol*37:1459-1463. [View Article]
5. Tanner MAC, Everett CL,Youvan(2000) Molecular phylogenetic evidence for noninvasive zoonotic transmission of Staphylococcus intermedius from canine pet to a human. *J Clin Microbiol* 38: 1628-1631. [View Article]
6. Van Duijkeren E, Wolfhagen MJHM, Box ATA, Heck MEOC, Wanet WJB, et al.(2004) Human-to-dog transmission of methicillin-resistant Staphylococcus aureus. *Emerg Infect Dis* 10: 2235-2237. [View Article]
7. Loeffler A, Boag AK, Sung J, Lindsay JA, Guardabassi L, et al.(2005) Prevalence of methicillin-resistant Staphylococcus aureus among staff and pets in a small animal referral hospital in the UK. *J Antimicrob Chemother* 56: 692-697. [View Article]
8. Weese JS, Archanbault M, Willey BM, Dick H, Hearn P, et al. (2005) Methicillin-resistant Staphylococcus aureus in horses and horse personnel, 2000-2002.*Emerg InfectDis* 11: 430-434. [View Article]
9. Strommenger B, Kehrenberg C, Kettlitz C, Cuny C, Verspohl J,et al.(2006) Molecular characterization of methicillin-resistant Staphylococcus aureus strains from pet animals and their relationship to human isolates. *J Antimicrob Chemother* 57: 461-465. [View Article]
10. Worthing A(2017)The Molecular Epidemiology of Antimicrobial Resistant Staphylococci in Australian Animals. Thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy. Sydney School of Veterinary Science. Faculty of Science The University of Sydney. [View Article]
11. Stickler DJ (2002)Susceptibility of antibiotic-resistant Gram-negative bacteria to biocides: a perspective from the study of catheter biofilms.*J Appl Microbiol* 92: 163S-170S. [View Article]
12. Mehrad B, Clark N, Zhanel G, Lynch J (2015) Antimicrobial Resistance in Hospital-Acquired Gram-Negative Bacterial Infections. *Chest* 147: 1413-1421. [View Article]
13. Division of Healthcare Quality Promotion (DHQP) (2001) Antimicrobial resistance prevention campaign. [presentación power point]. Atlanta, Georgia, USA. Centers for Disease Control and Prevention (CDC). 63 *diapositivas*. [View Article]
14. Calfee D(2012) Methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci, and other Gram-positives in healthcare. *Curr Opin Infect Dis* 25: 385-94. [View Article]
15. Fraise AP(2002) Susceptibility of antibiotic-resistant cocci to biocides. *J Appl Microbiol* 92: 158S-162. [View Article]
16. Russell AD(2002a) Introduction of biocides into clinical practice and the impact on antibiotic-resistant bacteria. *J Appl Microbiol* 92: 121S-135S. [View Article]
17. Silver S, Wendt L (1967) Mechanism of action of phenylethyl alcohol: breakdown of the cellular permeability barrier. *JBacteriol* 93: 560-566. [View Article]
18. McMurry LM, Oethinger M, Levy SB(1998) Overexpression of marA, soxS or acrAB produces resistance to triclosan in laboratory and clinical strains of Escherichia coli. *FEMS Microbiology Letters*. 166: 305-309. [View Article]
19. McMurry LM, Mcdermott PF, Levy SB(1999) Genetic evidence that InhA of Mycobacterium smegmatis is a target for triclosan. *Antimicrob Agents Chemother* 43: 711-713. [View Article]
20. Russel AD (200b2)Antibiotic and biocide resistance in bacteria: comments and conclusions. *J ApplMicrobiol* 92: 171S-173S. [View Article]
21. MaillardJy(2002) Bacterial target sites for biocide action. *J Appl Microbiol* 92: 16S-27S. [View Article]
22. Strong L, Gould T,Kasinkas L, Sadowsky M, Aksan A,et al.(2013) Biodegradation in waters from hydraulic fracturing: Chemistry, microbiology, and engineering. *J EnvironEng*140: 1-8. [View Article]
23. Jenkinson H(1996) Ins and Outs of Antimicrobial Resistance: Era of the Drug Pumps. *J Dent Res*75: 736-742. [View Article]

24. Houghteling P, Walker W(2015) Why is initial bacterial colonization of the intestine important to infants' and children's health? *J Pediatr gastroenterolnutr* 60: 294-307. [View Article]

25. Abraham E and Chain E (1940) An enzyme from bacteria able to destroy penicillin. *Nature* 146: 837-839. [View Article]

26. Poole K(2005) Efflux-mediated antimicrobial resistance. *J Antimicrob Chemother* 56: 20-51. [View Article]

27. Cabrera CE, Gómez RF,Zúñiga AE(2007) La resistencia de bacterias a antibióticos, antisépticos y desinfectantes, unamanifestación de los mecanismos de supervivencia y adaptación. *Colomb Med* 38: 149-158. [View Article]

28. Birt D, Boylston T, Hendrich S, Jane JL, Hollis J, et al.(2013) Resistant starch: promise for improving human health. *Adv nutr* 4: 587-601. [View Article]

29. Westh H, Zinn CS, Rosdahl VT, Sarisa Study Group(2004)An international multicenter study of antimicrobial consumption and resistance in Staphylococcus aureus isolates from 15 hospitals in 14 countries. *Microb Drug Resist* 10: 169-176. [View Article]

30. Sasaki T, Kikuchi K, Tanaka Y, Takahashi N, Kamata Set al. (2007) Methicillin-resistant Staphylococcus pseudintermedius in a veterinary teaching hospital.*J Clin Microbiol*45:1118-1125. [View Article]

31. Fair R and Tor Y(2014) Antibiotics and bacterial resistance in the 21st century. *Perspectmedicinchem* 6: 25-64. [View Article]

32. Bjorland J, Sunde M, SteinarWaage S (2001) Plasmid-borne smr gene causes resistance to quaternary ammonium compounds in bovine Staphylococcus aureus. *J Clin Microbiol* 39: 3999-4004. [View Article]

33. Blanco P, Hernando-Amado S, Reales-Calderon JA, Corona F, Lira F, et al.,(2016) Bacterial Multidrug Efflux Pumps: Much More Than Antibiotic Resistance Determinants. *Microorganisms* 4: 14-21. [View Article]

34. Goldstein C, Lee MD, Sánchez S, Hudson C, Phillips B, et al. (2001) Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother* 45: 723-736. [View Article]

35. Sunde M, Nostrom M (2006) The prevalence of associations between and conjugal transfer of antibiotic resistance genes in Escherichia coli isolated from Norwegian meat and meat products. *J Antimicrob Chemother* 58: 741-747. [View Article]

36. Deng Y, Bao X, Ji L, Chen L, Liu J, et al.(2015) Resistance integrons: class 1, 2 and 3 integrons. *Ann clin microbiolantimicrobi* 14: 45-51. [View Article]

Citation: Céspedes PF, Jara MA, Navarro CO (2018) Detection of Resistance Genes to Biocides in Bacteria Nosocomiales by Means the Chain Reaction of the Polymerase. Vet Sci Med 1: 001-006.