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# The Role of Mobile Genetics Elements in the Emergence of Superbugs.

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## ABSTRACT

**Background:** researchers have found that: *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and *Enterobacter* have acquired gene for gain and spared antibiotic resistance and known as "superbugs". Some of these chromosomally-encoded genes can be considered as part of the "core genome", whereas some other chromosomally-encoded genes are part of the "accessory genome" along with mobile genetic elements such as plasmids, bacteriophages, transposons and integrons. **Objective:** In this study we review the role that mobile genetic elements have played in pathogenic bacteria evolution, particularly in antibiotic resistance. **Results:** transposon and plasmid have played a noticeable role in forming the antibiotic resistance ability by carrying numerous number of antibiotics resistance genes such as genes encoding resistance to aminoglycoside and *β*-lactam. **Conclusion:** Mobile Genetic Elements (MGEs) play an important role in the evolution of a wide range of bacteria and are involved in the distribution of variable genes, such as virulence and antibiotic resistance genes causing innovation of 'hospital superbugs'.

**KEYWORDS:** antibiotic resistance, superbugs, mobile genetic elements, plasmids, bacteriophages, transposons

## INTRODUCTION

"Horizontal genomics" is a new area of prokaryotic biology that investigates DNA sequences present in the chromosome that appear to have originated from other prokaryotes or eukaryotes. Plasmids, bacteriophages and transposons encode the capability to mobilise from one host to another [1]. Qin, Galloway-Pena *et al* stated that the gain of mobile genetic elements carrying antibiotic resistance, virulence and/or fitness factors are the driving force behind the recent success of the superbugs [2]. Investigations of gene clusters that are associated with vancomycin resistance and methicillin resistance reported that horizontal gene transfer occurs between prokaryotes [3,4,5]. In addition, the *esp* virulence gene is located on a large pathogenicity-associated island in *E. faecium* and this *esp* PAI can be transferred horizontally and inserts in a site-specific manner [6,7]. MGEs are transferred to human isolates and thereby add to the burden of the disease caused by *E. faecium*, for example, by transferring vancomycin resistance between bacteria. This capability is important to consider, since these genes were shown to be transferred to human isolates and to more virulent organisms such as *Staphylococcusaureus*[2].A study by Call *et al.* (2010) analysed five *E. coli* and *Salmonella* plasmids carrying gene (*bla*CMY-2) encodes an AmpC-type beta-lactamase that hydrolyzes third-generation cephalosporins and represent share a common ancestor with the *Yersinia* and *Photobacterium* plasmids [8]. Although bacteriophages carrying antibiotic resistance genes have been identified in most of pathogenic bacteria [9, 10]. Their function in the distribution of virulence factors and antibiotic resistance genes has been widely recognised [11]**.** In this study we will focus on the mobile genetics element of the five riskiest superbugs which are *Enterococcus*, *Staphylococcus*, *Klebsiella*, *Acinetobacter*, *Pseudomonas* and *Enterobacter*to identify the role of mobile genetics element in evolution of antibiotic resistant bacteria that invading communities worldwide.

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## MATERIAL AND METHOD

*Plasmid identification:*

Plasmid genomes were obtained from the sequence of their hosts that were available from the NCBI database and antibiotics resistant encoding genes were predicted from these genomes of the plasmid by the genebank annotation.

*Sequence clustering and phylogenetics:*

Mauve progressive alignments to determine conserved sequence segments most likely to be conserved in recombination events were determined using the Mauve algorithm. The guide trees of several-selected plasmid were constructed with Mauve and tree was visualized using FigTree.

## RESULTS AND DISCUSSION

*Mobile genetics elements:*

*Transposon (Tn):*

Many transposons have been described in the riskiest superbugs that carrying genes encoding resistance to antimicrobials (Table1). Genes encoded resistance to Tetracycline, Vancomycin, Erythromycin, gentamicin, and mercuric chloride were found carried by transposon in *Enterococcus*.Streptogramin, Macrolide-lincosamide-SGB ($MLS_B$) were carried by Tn*5406*, Tn*554 and* Tn*3853*, respectively in *staphylococcus.* In*Klebsiella*Tn*1331 and* Tn*4401* has *stated to carry* Amikacin, Ampicillin, Kanamycin, Streptomycin, Tobramycin and β-lactamases. Resistance genes that known almost resistant to all antibiotics were found in Tn*AbaR1* in *Acinetobacter*.Aminoglycoside and beta lactam antibiotics were encoded by Tn*1331* that carried by *Enterobacter.* Tn*1545* andTn*916* both play an important role in the resistant to Tetracycline.

*Plasmids:*

Many plasmids have been described in superbug'sspecies that confer resistance to antimicrobials and heavy metals. According to the National Center for Biotechnology Information NCBI *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and*Enterobacter*, which known as the riskiest superbugs, have high number of plasmids (Table 2).

Within the species of *Enterococcus*, nighty five plasmids were identified and most of these plasmids were found in *Enterococcus faecalis* (twenty seven plasmids) and *Enterococcus faecium* (fifty three plasmids), these two species have become an increasing medical concern by their ability to gain and spread antibiotic resistance. Three hundred and nighty plasmids were identified in *Staphylococcus species*, andmost of these plasmids were carrying by *Staphylococcus aureus* strains (two hundred and sixty seven plasmids), which is methicillin-resistant (MRSA) and it is a worldwide problem in clinical medicine nowadays.

**Table 1:** The appearance of antibiotic resistance genes carried by transposon in six of the most important superbugs (*Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and*Enterobacter).*

| Superbugs | Tn | Antibiotic resistance carrying by the Tn |
|---|---|---|
| *Enterococcus* | Tn*1545*<br>Tn*916*<br>Tn*5397*<br>Tn*1549*<br>Tn*5385* | Tetracycline resistance [12]<br>Tetracycline resistance [13]<br>Tetracycline resistance [13]<br>Vancomycin Resistance [14]<br>Erythromycin, gentamicin, and mercuric chloride [15] |
| *Staphylococcus* | Tn*5406*<br>Tn*554*<br>Tn*3853* | Streptogramin [16]<br>Macrolide-lincosamide-SGB ($MLS_B$) antibiotics[16]<br>Macrolide-lincosamide-SGB ($MLS_B$) antibiotics[16] |
| *Klebsiella* | Tn*1331*<br>Tn*4401* | Amikacin, ampicillin, kanamycin, streptomycin, and tobramycin [17]<br>β-lactamases [18] |
| *Acinetobacter* | Tn*AbaR1* | 45 resistance genes and almost resistant to all antibiotics |
| *Pseudomonas* | Tn*1545*<br>Tn*916* | Tetracycline resistance [19]<br>Tetracycline resistance [19] |
| *Enterobacter* | Tn*1331* | Aminoglycoside and beta lactam antibiotics [20] |

*Klebsiella*species harbour more than three handers and seventy plasmids and about three hundred forty plasmids were identified in *Klebsiella pneumonia* which has a death rate around 50%, even with antimicrobial therapy and new antibiotic-resistant strains of *K. pneumoniae* are emerging [20, 21]. Nearlyone hundred forty from two hundred *Acinetobacter* plasmids were found in *Acinetobacterbaumannii strains.* It has documented to be amongst the most difficult multidrug-resistant gram-negative bacilli to treat and control [22]. *Pseudomonas* and *Enterobacter*harbournighty five and eighty-eight plasmids mostly in Pseudomonas *aeruginosa*,which is a prototypical "multidrug resistant (MDR) pathogen", and *Enterobactercloacae* that known as a member of the

normal gut flora [23]**(**Table 2).

To characterise the plasmid complement of these bacteria *in silico* the plasmids were annotated to identify whether or not these plasmids encoded antibiotic resistance genes. In addition, a comparative analysis was made with the plasmid sequences that were publicly available. Analysis of plasmid genome content across all of the publicly available genomes of the six most problematic superbugs that have became increasing medical concerned revealed relationships based on shared DNA sequences (Figure 1).

**Table 2:** The appearance of antibiotic resistance genes carried by plasmid in six of the most important superbugs (*Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and*Enterobacter).*

| Superbugs | No of Plasmids | Antibiotic resistance genes carrying by the plasmids |
|---|---|---|
| *Enterococcus* | 95 | Tetracycline, Streptothricin, Vancomycin, Erythromycin, Cytolysin, Chloramphenicol, florfenicol, Tigecycline, Daptomycin, Kanamycin, Streptomycin and Teicoplanin. |
| *Staphylococcus* | 390 | Vancomycin, Mupirocin, Neomycin, Tetracycline, Chloramphenicol, Streptomycin, Spectinomycin, Macrolide, Lincosamide, Streptogramin A and B, Fosfomycin, Pleuromutilin, Trimethoprim, Apramycin, Phenicols, Oxazolidinones, Erythromycin, Gentamicin, Apramycin, Methicillin, Bleomycin, Antiseptic, Isoleucine, Pleuromutilins, Kanamycin, Trimethoprim, Minocycline and Penicillins. |
| *Klebsiella* | 370 | Sulfonamide, Trimethoprim, Quinolone, Acriflavin, Glyoxalase, Bleomycin Tetracycline, Gentamicin, Tobramycin, Amikacin, Streptomycin, Colicin, Tunicamycin, Chloramphenicol,Fosfomycin, Florfenicol, Erythromycin, kanamycin, Neomycin, Apramycin, Trimethoprim, Rifampin and Spectinomycin |
| *Acinetobacter* | 212 | Sulfonamide, Trimethoprim, Aminoglycoside,Gentamicin, Bleomycin, Carbapenem, Amikacin, Kanamycin and Neomycin. |
| *Pseudomonas* | 97 | Sulfonamide, Quinolone, Streptomycin, Spectinomycin, Aminoglycoside, Gentamycin, Penicillin, Tetracycline, Trimethoprim, Beta-lactams and Glyoxalase/Bleomycin. |
| *Enterobacter* | 94 | Trimethoprim, Carbapenem, Quinolone, Sulfonamide, Tetracycline, Streptomycin, Beta lactamase, Aminoglycosids, Gentamicin, Tobramycinm, Chloramphenicol,Metallo-beta-Lactamase, Acriflavin, and Glyoxalase/Bleomycin. |

From the comparative analysis of the plasmids most of the plasmids isolated from specific species were grouped together. Clade A contains different range of plasmids from gram negative species only (*Enterobacter*, *Acinetobacter*, *Pseudomonas* and *Klebsiella*) which indicated that plasmids grouped in this clade are gram negative specific plasmids and could be transfer from one species to another. However, *Staphylococcus* plasmid revealed relationships with plasmids from gram positive and gram negative bacteria (*Enterococcus* and *Acinetobacter*) which indicate that *Staphylococcus, Enterococcus* and *Acinetobacter* share similar types of plasmids (clade C and D). *Acinetobacter* harbor two different types of plasmid grouped in clade B and D. *Acinetobacter* plasmids in clade B seem to be specific to this species (Figure 1).

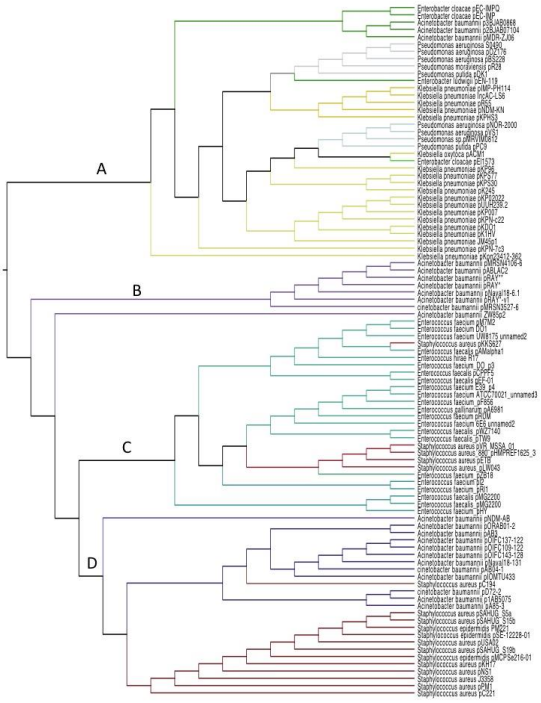


**Fig. 1:**Cladogram tree of the superbug's plasmids *Enterococcus, Staphylococcus, Klebsiella, Acinetobacter, Pseudomonas* and *Enterobacter).* The tree is based on an alignment of the amino acid sequence of 95 plasmids.

Disease treatment and growth promotion could explain the multiple antimicrobial resistances of most superbugs, including animal strains. The delivery of low levels of antimicrobials has apparently resulted in considerable colonisation of animals with antibiotic resistant bacteria, such as *E. coli* strains and acquisition of resistance in *E. coli* in the intestinal flora of the farmers has been described [24, 25]. Aarestrup (2000) reported that resistance to streptothricin antibiotics has been described in Gram-negative bacteria as a result of using nourseothricin as an antimicrobial feed promoter in industrial animal farms in Germany. In addition, resistance to streptogramins may be related to the use of virginamycin, as a feed promoter combined in agriculture for animal food production [26].In anther hand, mobile genetics elements play an important role of the emergence and the spread of multidrug resistance bacteria. "Horizontal genomics" is a new area of prokaryotic biology that investigates DNA sequences present in the chromosome that appear to have originated from other prokaryotes or eukaryotes. Plasmids, bacteriophages and transposons encode the capability to mobilise from one host to another [27]. Galloway-Pena *et al* (2012) stated that the gain of mobile genetic elements carrying antibiotic resistance, virulence and/or fitness factors are the driving force behind the recent success of superbugs as an opportunistic pathogen in hospitals. Investigations of gene clusters that are associated with vancomycin resistance and Tn*1546* in *E. faecium*, reported that horizontal gene transfer occurs between human and animal *E. faecium*isolates [2, 3, 4, 5]. MGEs are transferred to human isolates and thereby add to the burden of the disease caused by *E. faecium*, for example, by transferring vancomycin resistance between bacteria. This capability is important to consider, since these genes were shown to be transferred to human isolates and to more virulent organisms such as *Staphylococcus aureus*[2].

Most research intended at antibiotic resistant bacteria has inspired on attacking the bacteria and developing new antibiotics, nevertheless, instead we have to come at the problem from the host such as observing ways the patient's immune system can be altered to prevent susceptibility to infection, attacking mobile genetics elements and mutations that known to encode antibiotics resistance as well as start a critical thinking of using medical plant and soil microbiom to produce new antibiotics. Targeting antibiotic resistant bacteria with CRISPR and phages can sensitize the microbes to the drugs, targeted killing and plasmid removal. The CRISPR can be designed to target unique sequences in the bacterial chromosome or in harbored plasmids. CRISPR antimicrobials can target specific sequences in a single virulent bacterial species, or even an antibiotic resistance gene. Another approach for CRISPR antimicrobials is to associate them with both replication-competent phages and antibiotics [28, 29]. These techniques open a wide range of applications for the delivery of CRISPR based antimicrobials that are otherwise poorly addressed by traditional antibiotics. Recently, data on the antimicrobial activity of several plants have been scientifically established, beside the cumulative number of studies on pathogenic microorganisms resistant to antimicrobials. Plants products may possibly control microbial growth in varied situations and in the certain case of disease treatment, several studies have considered to define the chemical composition of plant antimicrobials and the mechanisms elaborate in microbial growth inhibition, either separately or connected to predictable antimicrobials[30].

*Conclusion:*

Using antimicrobials as growth promoters in livestock production and the typical treatment for bacterial infections which is a bactericidal and synergistic mixture of a cell wall synthesis inhibitor such as a β-lactam antibiotic or glycopeptide, with an aminoglycoside has been described as reasons of the emergence of bacteria with high-level resistance The typical treatment for bacterial infections is a bactericidal and synergistic mixture of a cell wall synthesis inhibitor such as a β-lactam antibiotic or glycopeptide, with an aminoglycoside. However, the efficacy of this combination has been compromised by the emergence of bacteria with high-level resistance. The increasing occurrence of multi-resistant pathogenic bacteria is formed a critical demand in the modern world for new rational approaches and strategies to the screening of antibiotics with an extensive spectrum of activity, that can resist the inactivation processes exploited by microbial enzymes.

## REFERENCES

1. Frost, L.S., R. Leplae, A.O. Summers and A. Toussaint, 2005. "Mobile genetic elements: the agents of open source evolution." Nat Rev Microbiol., 3(9): 722-732.
2. Groopman, J., 2008. "Superbugs: The new generation of resistant infections is almost impossible to treat." The New Yorker 2008-08-11.
3. Qin, X., J.R. Galloway-Pena, J. Sillanpaa, J.H. Roh, S.R. Nallapareddy, S. Chowdhury, A. Bourgogne, T. Choudhury, D.M. Muzny, C.J. Buhay, Y. Ding, S. Dugan-Rocha, W. Liu, C. Kovar, E. Sodergren, S. Highlander, J.F. Petrosino, K.C. Worley, R.A. Gibbs, G.M. Weinstock and B.E. Murray, 2012. "Complete genome sequence of Enterococcus faecium strain TX16 and comparative genomic analysis of Enterococcus faecium genomes." BMC Microbiol., 12: 135.
4. Stobberingh, E., A. van den Bogaard, N. London, C. Driessen, J. Top and R. Willems, 1999. "Enterococci with glycopeptide resistance in turkeys, turkey farmers, turkey slaughterers, and (sub)urban residents in the

south of The Netherlands: evidence for transmission of vancomycin resistance from animals to humans?" Antimicrob Agents Chemother., 43(9): 2215-2221.
5. van den Bogaard, A.E., R. Willems, N. London, J. Top and E.E. Stobberingh, 2002. "Antibiotic resistance of faecal enterococci in poultry, poultry farmers and poultry slaughterers." J Antimicrob Chemother, 49(3): 497-505.
6. Willems, 2010. "Pyrosequencing-based comparative genome analysis of the nosocomial pathogen Enterococcus faecium and identification of a large transferable pathogenicity island." BMC Genomics, 11: 239.
7. Leavis, H., J. Top, N. Shankar, K. Borgen, M. Bonten, J. van Embden and R.J.L. Willems, 2004. "A Novel Putative Enterococcal Pathogenicity Island Linked to the esp Virulence Gene of Enterococcus faecium and Associated with Epidemicity." Journal of Bacteriology, 186(3): 672-682.
8. van Schaik, W., J. Top, D.R. Riley, J. Boekhorst, J.E. Vrijenhoek, C.M. Schapendonk, A.P. Hendrickx, I.J. Nijman, M.J. Bonten, H. Tettelin and R.J.Yasmin, A., J.G. Kenny, J. Shankar, A.C. Darby, N. Hall, C. Edwards and M.J. Horsburgh, 2010. "Comparative genomics and transduction potential of Enterococcus faecalis temperate bacteriophages." J Bacteriol., 192(4): 1122-1130.
9. Call, D.R., R.S. Singer, D. Meng, S.L. Broschat, L.H. Orfe, J.M. Anderson, D.R. Herndon, L.S. Kappmeyer, J.B. Daniels and a.T.E. Besser, 2010. "blaCMY-2-positive IncA/C plasmids from Escherichia coli and Salmonella enterica are a distinct component of a larger lineage of plasmids." Antimicrob. Agents Chemother, 54:590-596.
10. Yasmin, A., J.G. Kenny, J. Shankar, A.C. Darby, N. Hall, C. Edwards and M.J. Horsburgh, 2010. "Comparative genomics and transduction potential of Enterococcus faecalis temperate bacteriophages." J Bacteriol., 192(4): 1122-1130.
11. Davies, J. and D. Davies, 2010. "Origins and evolution of antibiotic resistance." Microbiol Mol. Biol. Rev., 74:417-433.
12. Boyd, E.F. and H. Brussow, 2002. "Common themes among bacteriophage-encoded virulence factors and diversity among the bacteriophages involved." Trends Microbiol., 10:521-529.
13. Doucet-Populaire, F., P.Trieu-Cuot, I.Dosbaa, A.Andremont and C.P., 1991. "Inducible transfer of conjugative transposon Tn1545 from Enterococcus faecalis to Listeria monocytogenes in the digestive tracts of gnotobiotic mice." Antimicrob Agents Chemother., 35(1):185-7.
14. Agersø, Y., A.G. Pedersen and F.M. Aarestrup, 2006. "Identification of Tn5397-like and Tn916-like transposons and diversity of the tetracycline resistance gene tet(M) in enterococci from humans, pigs and poultry." Journal of Antimicrobial Chemotherapy, 57: 832-839.
15. Aline Launay, Susan A. Ballard, Paul D.R. Johnson, M.L. Grayson and Thierry Lambert, 2006. "Transfer of Vancomycin Resistance Transposon Tn1549 from Clostridium symbiosum to Enterococcus spp. in the Gut of Gnotobiotic Mice." Antimicrob Agents Chemother, 50(3): 1054-1062.
16. ME, B., L.L.Carias and L.B.R., 1997. "Enterococcal transposon Tn5384: evolution of a composite transposon through cointegration of enterococcal and staphylococcal plasmids." Antimicrob Agents Chemother., 41(9):1854-8.
17. Julien Haroche, J. Allignet and N.E. Solh* 2002. "Tn5406, a New Staphylococcal Transposon Conferring Resistance to Streptogramin A and Related Compounds Including Dalfopristin.
18. Tolmasky, M.E., R.M. Chamorro, J.H. Crosa and P.M. Marini, 1988. "Transposon-mediated amikacin resistance in Klebsiella pneumoniae." Antimicrob Agents Chemotherv, 32(9): 1988.
19. Munoz-Price, L.S. and J.P. Quinn, 2009. "The Spread of Klebsiella pneumoniae Carbapenemases: A Tale of Strains, Plasmids, and Transposons." Oxford JournalsMedicine & Health Clinical Infectious Diseases 49(11).
20. Poyart, C.J.C. and P. Trieu-Cuot, 1995. "Conjugative transposition of Tn916-related elements from Enterococcus faecalis to Escherichia coli and Pseudomonas fluorescens." Antimicrob Agents Chemother., 39(2): 500-506.
21. Mohammad, R Alavi, VladoAntonic, AdrienRavizee, Peter J Weina, Mina Izadjoo and A. Stojadinovic, 2011. "An Enterobacter plasmid as a new genetic background for the transposon Tn1331." Infect Drug Resist. 4: 209-213.
22. Groopman, J., 2008. "Superbugs: The new generation of resistant infections is almost impossible to treat." The New Yorker 2008-08-11.
23. Maragakis, L.L. and T.M. Perl, 2008. "Acinetobacter baumannii: epidemiology, antimicrobial resistance, and treatment options." Clin Infect Dis., 46(8): 1254-1263.
24. Gill, Sunil Arora, J.S., S.P. Khanna and K.H. Kumar, 2016. "Prevalence of multidrug-resistant, extensively drug-resistant, and pandrug-resistant Pseudomonas aeruginosa from a tertiary level Intensive Care Unit." Journal of Global Infectious Diseases, 8(4): 155-159.
25. Marshall, B.M. and S.B. Levy, 2011. "Food animals and antimicrobials: impacts on human health." Clin Microbiol Rev., 24(4): 718-733.

26. Lebreton, F., W. van Schaik, A.M. McGuire, P. Godfrey, A. Griggs, V. Mazumdar, J. Corander, L. Cheng, S. Saif, S. Young, Q. Zeng, J. Wortman, B. Birren, R.J. Willems, A.M. Earl and M.S. Gilmore, 2013. "Emergence of epidemic multidrug-resistant Enterococcus faecium from animal and commensal strains." MBio 4(4).
27. Aarestrup, F.M., 2000. "Characterization of glycopeptide-resistant enterococcus faecium (GRE) from broilers and pigs in Denmark: genetic evidence that persistence of GRE in pig herds is associated with coselection by resistance to macrolides." J Clin Microbiol., 38(7): 2774-2777.
28. Frost, L.S., R. Leplae, A.O. Summers and A. Toussaint, 2005. "Mobile genetic elements: the agents of open source evolution." Nat Rev Microbiol., 3(9): 722-732.
29. Beisel, C.L., A.A. Gomaa and R. Barrangou, 2014. "A CRISPR design for next-generation antimicrobials." Genome Biol., 15(11): 516.
30. Yosef, I., M. Manor, R. Kiro and U. Qimron, 2015. "Temperate and lytic bacteriophages programmed to sensitize and kill antibiotic-resistant bacteria." Proc Natl Acad Sci U S A 112(23): 7267-7272.
31. NCC, S. and F. Júnior, 2010. "Biological properties of medicinal plants: a review of their antimicrobial activity." The Journal of Venomous Animals and Toxins including Tropical Diseases., 16(3): 402-413.