

Molecular Characterization of *Tuf* Gene and Antibiotic Susceptibility Patterns of *Streptococcus* Species in Local Population of Lahore, Pakistan

A. Mahmood^{1,2}, A. J. Sami^{1*} and Q. Ali²

¹Institute of Biochemistry and Biotechnology, University of the Punjab, Lahore, Pakistan

²Institute of Molecular Biology and Biotechnology, The University of Lahore, Pakistan

Corresponding author: Amtul Jamil Sami

E-mail: amtuljamilsami@yahoo.com

Genet. Mol. Res. 19 (4): gmr16039988

Received: August 03, 2020

Accepted: August 17, 2020

Published: August 24, 2020

Copyright © 2018 The Authors. This is an open-access article distributed under the terms of the Creative Commons Attribution ShareAlike (CC BY-SA) 4.0 License.

ABSTRACT.

Background: Multi Drug Resistance (MDR) is a global problem which is expeditiously increasing. Different antimicrobial drugs have been reported resistant against various *Streptococcus* species. This has been a threat to local individuals in all over the world specifically in developing countries including Pakistan. Decreased efficiency of drugs due to emergence of new resistance mechanisms in treatment of common infectious diseases like pneumonia and meningitis has been increasing. The cause of the failure of microbial response to standard treatment leading to prolonged illness and high risk of death. This study aims to determine the impact of antibiotics on *Streptococcus* species by studying the resistance and sensitivity patterns in local population of Lahore. **Methods:** A community based study has been conducted from different areas of Lahore. A total of 220 clinical samples including oral dental caries, pus, nasopharyngeal, urine and skin has been studied from June 2018 to May 2019. Species identification has been done by microscopic visualization, Gram staining and biochemical identification including catalase, coagulase, bile esculin and NaCl broth test. Antibiotic susceptibility patterns of the clinical isolates have been defined by using commercially prepared antibiotic discs (Streptomycin, Ampicillin, Vancomycin, Oxacillin, Tetracycline,

Clindamycin, Chloramphenicol, Gentamicin, Amoxicillin and Azithromycin). Molecular characterization of resistant *Streptococcus* species has been evaluated by targeting *tuf* gene. Primers have been designed manually using consensus gene sequence of *tuf* gene of *Streptococcus* species from PUBMED and Primer BLAST has been used to analyse the quality of primers. DNA was isolated by phenol Chloroform Method. Polymerase Chain Reaction (PCR) was performed to amplify *tuf* gene. The amplified product has been evaluated by using Agarose Gel Electrophoresis. **Results:** Resistance and Sensitivity have been studied against *Streptococci* according to the mode of the action of different antibiotics. It was observed that resistance of *Streptococcus* species against the specific drugs is increasing gradually. Exceptionally surprising results have also been observed as some of the species were sensitive to different antibiotics in previously reported studies but currently become resistant. **Conclusion:** This study concluded *Streptococcus* species including *S. mitis* and *S. bovis* are opportunistic, upon getting the required conditions these become infectious. Malnutrition is one of the major causes of the increasing resistance patterns of *Streptococcus* species.

Keywords: Multi drug resistance; *Streptococcus*; Antibiotic; *Tuf* gene; Gel electrophoresis

INTRODUCTION

Numerous microorganisms almost greater than 100 species, which occur in the mucous membranes of humans as well as animals, are categorized under the *Streptococcus* genus. They are present in the form of natural flora occupying the oral cavity as well as the intestines of animals and humans. Additionally they usually occur on the skin, upper respiratory tract as well as in the throat. Various Streptococci occur as expedient pathogens and cause numerous diseases in the host body which lacks immunological responses (Cole et al., 2008). Microorganisms that are believed to cause most of the common infections include *S. pneumoniae*, *S. pyogenes*-group A *Streptococcus*, *S. agalactiae*-group B *Streptococcus* and *S. mutans* (Cole et al., 2008; Miftahussurur et al., 2016). Over the past decades, a rapid increase in the number of antibiotic-resistant clinical isolates has been increased rapidly while the rates of development and origination of new antimicrobials have been observed to be comparatively very low. These two conditions collectively pose a great threat to human health, as the sustenance of these conditions may lead to the prevalence of bacterial pathogens due to no treatment present against them (Tettelin et al., 2001; Tong et al., 2015). This condition would result in increased morbidity and mortality affiliated with the infectious diseases and this state could reach levels higher than those seen in the pre antibiotic period. Microbes possess an extraordinary capability to develop resistance and this can be easily understood when observed through the evolutionary perspective. Along with the use of natural antimicrobials, mechanisms of bacterial resistance have also been coevolving for billions of years. The therapeutic use of antibiotics started about 70 years ago (Ali et al., 2020; Danish et al., 2020; Garau et al., 2005). In addition to this, resistant strains are usually less virulent and resultantly less competitive than the sensitive strains from which they originated. Due to this reason, resistance was less propagated (Khalil et al., 2020ab; Levy et al., 2004).

After the first antibiotic was introduced into the medical practice, emergence of antibiotic resistance was also observed alongside. The resistant microorganisms showed resistance towards other classes of antibiotics as well, and this resistance occurred without any previous exposure of the microbes to these antibiotics. This was the indication of multiple resistances developed by the organisms against a wide range of antibiotics. This multiple resistance was also termed as Multi Drug Resistance (MDR). MDR phenomenon was initially observed in

mammalian cells after the observation of its molecular basis of these cells (Mushtaq et al., 2020; Fernández and Hancock, 2012). Among the well-known causes of MDR, efflux pumps are considered to be the major cause. The multidrug efflux phenomenon leads to the bacterial resistance towards the valuable clinical antibiotics and therefore is identified as a significant mechanism. Bacteria possessing the MDR phenomenon also show resistance against cytotoxic agents which they are never exposed to, and this activity is considered to pose a great terror for drug-based clinical treatments (Mika et al., 2015; Rasmussen et al., 2000; Von et al., 2001). Bacteria display a vast variety of mechanisms for resisting the pathogenic activities. These mechanisms are often found to be specified against an individual drug or against a variety of drugs that are correlating with each other. An example of such mechanism is Beta-Lactamase, which performs the function of hydrolysing the beta-lactam ring of antibiotics (Walsh, 2000).

Mutational modification of the drug target is another well-known mechanism of resistance. This mechanism works by reducing the affinity of the target for the drug or to reducing the permeability of the cell envelope (Walsh, 2000). In gram-negative bacteria and mycobacteria, this reduction in the permeability can also be obtained by reducing the expression of porins. Porins are protein in nature and the function of these proteins is to assist the entry of smaller molecules by serving as entry gates in specific nutrients. Along with this, they also assist in the transport of pathogens (Fernández and Hancock, 2012; Magiorakos et al., 2012). The antibiotic families including Macrolides (Clindamycin, and Azithromycin), amino penicillin (Ampicillin), Penicillinase Resistant (Oxacillin) Aminoglycosides (Gentamycin and streptomycin), Beta Lactam (Amoxicillin), Glyco-peptide antibiotics (Vancomycin) chloramphenicol and tetracycline. Aminoglycosides are bactericidal and inhibit protein synthesis by binding 30-S ribosomal subunit. Tetracyclines are bacteriostatic and blocks tRNA. Macrolides are bacteriostatic and reversibly to bind to 50 S subunit.

MATERIALS AND METHODS

Sample collection

The Clinical samples were collected from different areas of Lahore. Clinical samples such as nasal, dental, oral and urine samples were included. Sterile swabs were used for the sample collection.

Sample screening

The samples brought were swabbed on the nutrient agar plates that were already prepared. These plates were then kept in the incubator overnight to provide the incubation period. Next day growth was observed which showed mixed colonies. From the mixed cultures obtained, separate colonies were picked up using an inoculum loop and were streaked on new plates of nutrient agar. These plates were also incubated overnight and growth was observed next day. The pure cultures were stored by either keeping the plates in the refrigerator or by preparing the stock solutions. The mixed culture/ were again purified if obtained (Martineau et al., 2001).

Identification of *Streptococcus* species

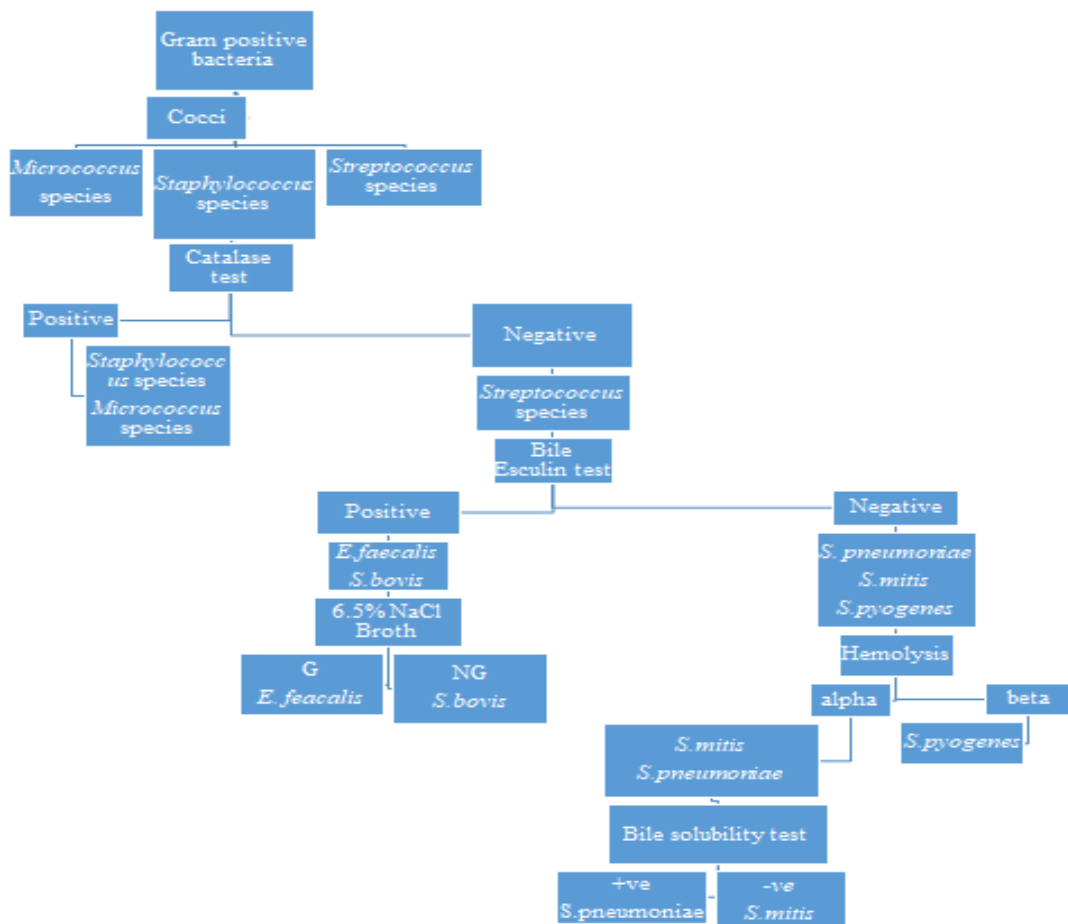
Streptococci give best growth on blood agar as compared to any other medium; therefore blood agar was used as a differential media for identification of *Streptococcus* species. Blood agar plates were prepared and samples were swabbed on the plates. Plates were then kept in the incubator overnight. Results were observed the next day.

Biochemical characterization

Catalase, coagulase, Gram staining, bile esculin and NaCl broth test. For the catalase test a drop of hydrogen peroxide (3%) was poured on a plain glass slide, then a small inoculum of bacterial isolate was picked up by a loop and mixed into the drop of hydrogen peroxide, slide was then observed. Bubble formation indicated a positive while no bubble formation indicated a negative result. *Streptococcus* species are Catalase negative. Bile Esculin test was performed on the samples which were catalase negative (Reiner, 2010). The bile esculin media

plates were prepared and samples were swabbed on it, after swabbing the plates were kept in the incubator for providing the incubation period of about 24 hours. The growth was observed next day. The samples which gave Bile Esculin positive results were separated from those which gave negative results. NaCl Broth test was performed for specie identification, on the samples which were Bile Esculin positive. The salt tolerance medium was prepared to differentiate the enterococcus species by taking into account the ability of *enterococci* to grow in the presence of 6.5% NaCl. The NaCl, BHI (brain and heart infusion), Dextrose, Bromocreserol Purple and Agar were used according to specific calculations, for the media preparation. Once the media plates were prepared, the bacterial colonies were picked up from pure cultures and were swabbed on these media plates. Plates were then kept in the incubator and growth was observed after 24 hrs. The plates which showed the bromocreserol purple turning to yellow were considered as positive test while the plates that showed no colour change were determined as negative test. The samples which gave bile esculin negative results were then grown on blood agar plates to check the hemolysis. After swabbing on blood agar plates the plates were left in the incubator for 24 hours. Next day growth was observed and species were identified on the basis of Alpha, Beta and Gamma hemolysis. The bile solubility test also called sodium deoxycolate solubility test was performed to differentiate the *Streptococcus pneumoniae* from other Streptococci showing the alpha hemolysis. This differentiation is possible because *Streptococcus pneumoniae* are bile soluble whereas all other *Streptococci* are bile resistant. Sodium deoxycolate causes the lysis of pneumococci cell wall. A clearing of turbidity occurs in the bile tube but does not in the saline control tube, a positive test result is indicated (Martineau et al., 2001) (Flow Chart 1).

Identification chart of *Streptococcus* species



Flow Chart 1. Identification chart of *Streptococcus* species.

Identification of *Streptococcus* species by PCR

Primers	AME	Sequence	Size of PCR product
Forward Primer	F <i>tuf</i>	5-ATGGTCCAATGCCACAAACACGTGAAC-3	354 bp
Reverse Primer	R <i>tuf</i>	5-CTACAGTACCACGACCAGTGATTG-3	

Table 1. *Streptococcus* species were identified by amplification of *tuf* gene (Martineau et al. 2001).

PCR amplification was performed in a 25 µl reactions containing 5 µl of microbial culture, 0.5 µM of forward and reverse primers (Table 1), and 12.5 µl of GoTaq Green Master Mix, (Promega, Madison, WI, USA), and nuclease free water. The forward and reverse primers specific for *tuf* gene have been designed manually using consensus gene sequence of *tuf* gene of *Streptococcus* species from PUBMED and Primer BLAST has been used to analyse the quality of primers. corresponding to yielding a 354-bp product. Amplification of *tuf* gene has been performed under the following conditions: one cycle of initial denaturation at 94°C for 4 min; 35cycles of 94°C for 30s, Annealing 62.8°C for 30s, extension at 72°C for 60 s; and one cycle of final extension at 72°C for 10 min. The amplified *tuf* gene was analysed using agarose gel electrophoresis.

Antibiotic susceptibility

Antibiotic susceptibility test was done to measure the action of different antibiotics on *Streptococcus* species and this susceptibility was measured by Kirby-Bauer disc diffusion method. All the process was performed according to Clinical Laboratory standards Institute (CLSI) reference method (Johnston and Jaykus, 2004). For this, the turbidity was checked by McFarland standard. 1% solution of barium chloride and sulphuric acid was prepared and combined it by mixing these two solutions to form turbid solution. The resulting mixture was placed in a foil covered screw cap tube (Bauer et al., 1959; Abera and Kibret, 2014). Muller hinton agar was used to test antibiotic susceptibility test. Media was prepared by adding the required quantity in distilled water in a sterile flask. This media was autoclaved and then placed media at room temperature to maintain the temp. Media was poured in petri plates and kept to solidify at room temperature. The petri plates was properly labelled and stored in refrigerator (Camara et al., 2013). Dilutions of McFarland standard were prepared in test tubes and properly labelled the test tubes. Bacteria were inoculated in each test tube by platinum loop and a suspension was prepared. Now 0.5 µl suspension was taken from each test tube and swabbed on Muller Hinton agar plates in three dimensional. Commercially prepared antibiotics (Gentamicin, Clindamycin, Amoxicillin, Ampicillin, Oxacillin, Chloramphenicol, Vancomycin, Azithromycin, Streptomycin and Tetracycline) were applied on these plates with the help of sterile forceps and incubation time was given (18-24 hrs.) at 37°C and then were examined for bacterial growth. Zone of inhibition were measured by callipers to check the sensitivity and resistivity of bacteria against drugs (Hudzicki, 2009; Johnston and Jaykus, 2004).

RESULTS

The cultures revealed different species of microorganisms including *S. aureus*, *S. epidermidis*, *B. cereus*, *B. subtilis*, *B. licheniformis*, *P. aeruginosa*, *K. pneumoniae*, *S. typhi*, *E. faecalis*, *S. bovis*, *S. mitis* and *S. pyogenes*. The presence of *Streptococcus* species was confirmed by Gram staining and different biochemical tests including catalase, coagulase, Bile solubility, bile esculin and NaCl broth test.

Susceptibility patterns of *Streptococcus* species

Antibiotic susceptibility test was performed on Muller Hinton agar. Ten different antibiotics were used against the isolated species which involved *E. faecalis*, *S. bovis*, *S. mitis* and *S. pyogenes*. Zones of inhibition of the antibiotics against these species were measured. Different combinations of antibiotics have been used to evaluate the resistance and sensitivity patterns of *Streptococcus* species. Among the strains of *Enterococcus faecalis*, Vancomycin, Azithromycin, Chloramphenicol, Streptomycin and Gentamycin were found highly susceptible where 100% strains were found sensitive against *E. faecalis*. *S. bovis* strains showed maximum sensitivity towards most of

the drugs. Vancomycin and Gentamycin found completely susceptible as 100% strains showed sensitivity towards it. *S. bovis* strains were found resistant to the clindamycin (83%) and Tetracycline (68%) respectively. *S. mitis* strains were found sensitive towards some of the drugs including Streptomycin, Vancomycin and Azithromycin. Gentamycin found completely susceptible as 100% strains showed sensitivity towards it. The strains were found completely resistant to Ampicillin, Amoxicillin and Oxacillin. Moreover the strains were moderately resistant towards Tetracycline, Clindamycin and Chloramphenicol. *S. pyogenes* were found completely resistant towards Streptomycin and Gentamycin as 100% strains showed resistance against these drugs. Azithromycin, Ampicillin, Amoxicillin, Clindamycin and Oxacillin were also found highly resistant. On the other hand these strains were completely sensitive towards Vancomycin and Chloramphenicol.

Prevalence of microbial flora

Percentage prevalence of microbial flora described to demonstrate how susceptible these species are in the local population of Lahore (Figure 1).

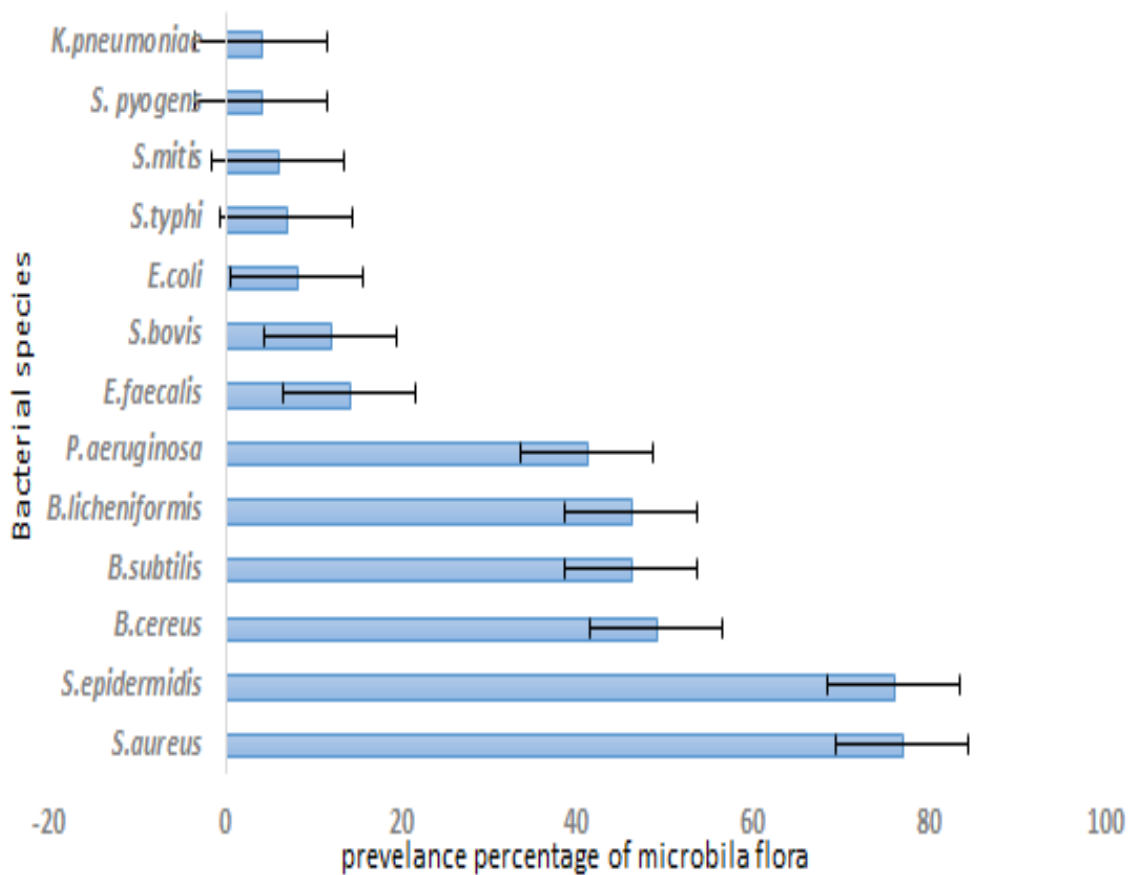


Figure 1. Prevalence of pathogenic microbes in clinical isolates in Lahore, Pakistan

Antibiotic susceptibility of *E. faecalis*

Among the strains of *Enterococcus faecalis*, Vancomycin, Azithromycin, Chloramphenicol, Streptomycin and Gentamycin were found highly susceptible where 100% strains were found sensitive against *E. faecalis*. Clindamycin and Amoxicillin also showed relatively higher sensitivity towards this specie. All other drugs were found to be moderately sensitive and resistant (Figure 2).

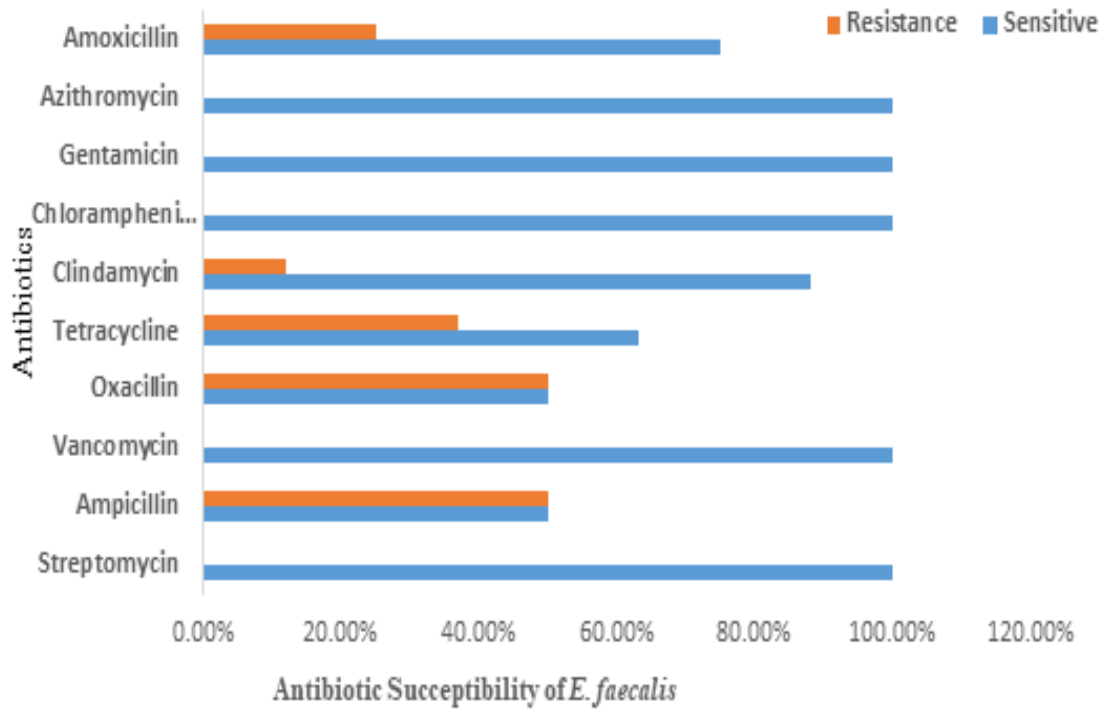


Figure 2. Susceptibility pattern of *E. faecalis* in local population of Lahore.

Antibiotic susceptibility of *Streptococcus bovis*

After testing the antibiotics against *Streptococcus bovis*, it was observed that *S. bovis* strains showed maximum sensitivity towards most of the drugs. Vancomycin and Gentamycin found completely susceptible as 100% strains showed sensitivity towards it. Other drugs including streptomycin, ampicillin, chloramphenicol and azithromycin were also highly susceptible as 83% strains showed sensitivity towards these drugs. Oxacillin and amoxicillin were found moderately susceptible. *S. bovis* strains were found resistant to the clindamycin (83%) and tetracycline (68%) respectively (Figure 3).

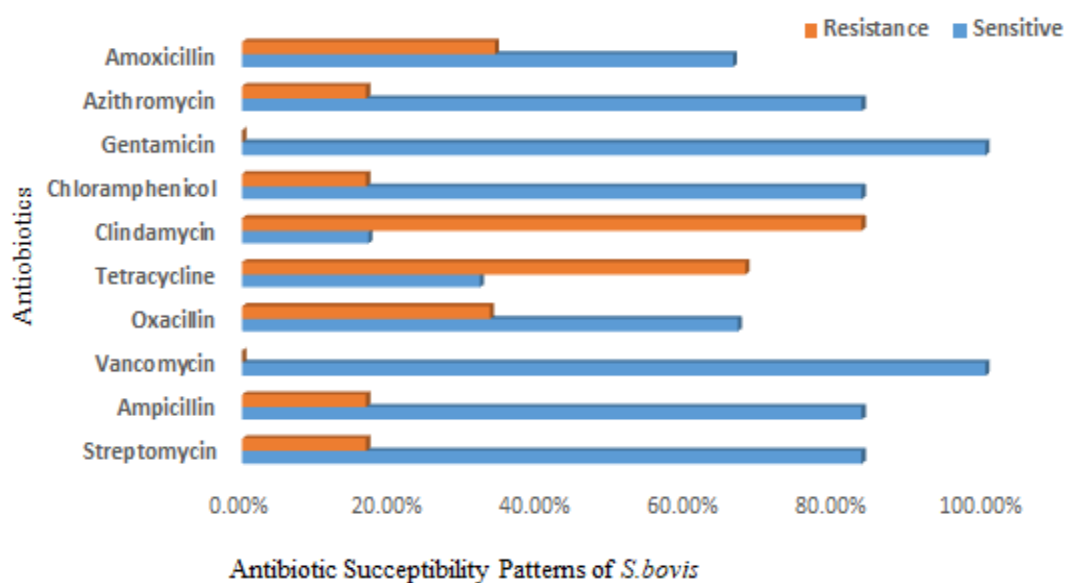


Figure 3. Susceptibility pattern of *Streptococcus bovis* in local population of Lahore.

Antibiotic susceptibility of *Streptococcus mitis*

S. mitis strains were found sensitive towards some of the drugs including streptomycin, vancomycin and Azithromycin. Gentamycin found completely susceptible as 100% strains showed sensitivity towards it. The strains were found completely resistant to Ampicillin, amoxicillin and oxacillin. Moreover the strains were moderately resistant towards tetracycline, clindamycin and chloramphenicol (Figure 4).

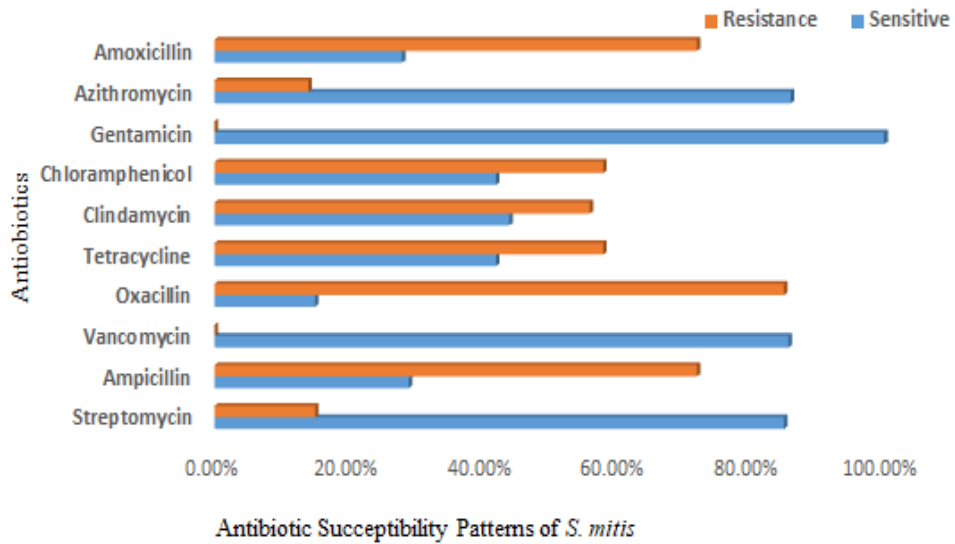


Figure 4. Susceptibility pattern of *Streptococcus mitis* in local population of Lahore.

Antibiotic susceptibility of *Streptococcus pyogenes*

S. pyogenes were found completely resistant towards streptomycin and Gentamycin as 100% strains showed resistance against these drugs. Azithromycin, Ampicillin, amoxicillin, clindamycin and oxacillin were also found highly resistant. On the other hand these strains were completely sensitive towards vancomycin and chloramphenicol (Figure 5).

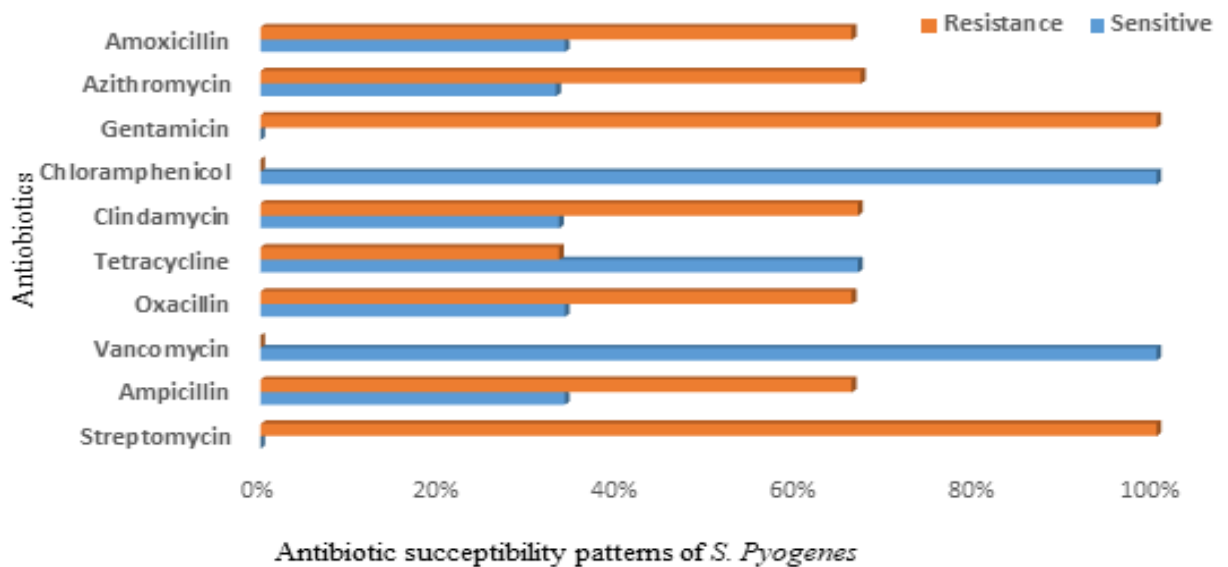


Figure 5. Susceptibility pattern of *Streptococcus pyogenes* in local population of Lahore.

PCR assay for *Streptococcus* species

The clinically isolated and biochemically confirmed *Streptococcus* species were characterized by Polymerase Chain Reaction (PCR) by amplifying a 354 bp *tuf* gene elongation factor specific to these species (Figure 6).

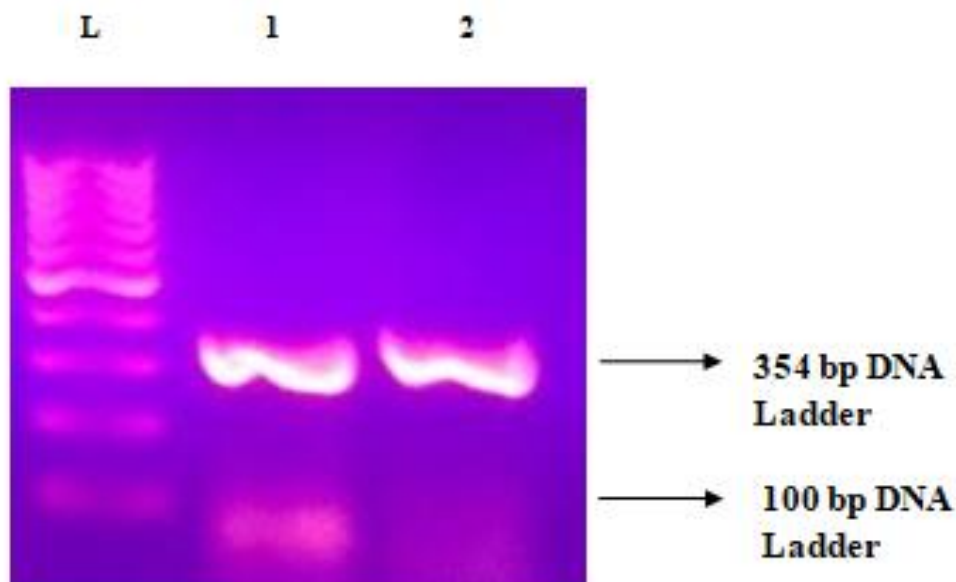


Figure 6. PCR amplification of a 354 bp fragment of *tuf* gene specific to *Streptococcus* species. Lane L, DNA Ladder; lanes 1 and 2 positive sample.

DISCUSSION

As per the current status of emerging resistance of bacterial species against antibiotics, the antibiotic susceptibility have been studied against certain species of the *Streptococcus* genus which included *Enterococcus faecalis*, *Streptococcus bovis*, *Streptococcus pyogenes* and *Streptococcus mitis*. The current work is an extension of a previous work done on *Staphylococcus* species among children in Lahore. (Sami et al., 2018), Resistance to Ampicillin, Oxacillin and Amoxicillin among clinical isolates of *S. mitis* group of *Streptococci* is increasingly recognized. In this study, a high prevalence of resistance to these drugs among *S. mitis* strains has been observed as almost 71%-85% strains within the range of (13 mm-14 mm). This result was in accordance with the results obtained by (Jans et al., 2015; Li and Nikaido 2004; Susan et al., 1999; Verhoeven et al., 2015) *S. mitis* strains were highly sensitive to Vancomycin 85% and Streptomycin 85% whereas moderate sensitivity of *S. mitis* was observed against Tetracycline 42%, Clindamycin 42%, Chloramphenicol 42% and Azithromycin 42%. This result was recorded on the basis of sensitivity range being (15 mm-23 mm). *S. mitis* showed highest sensitivity towards Gentamycin 100% and these results were in agreement with the findings of (Abera and Kibret, 2014). In this study, Streptomycin and Gentamycin were highly resistant to *S. pyogenes*, giving 100% resistant strains according to the range (13 mm-14 mm). Ampicillin, Amoxicillin and Oxacillin also showed greater resistance 66% and this result did not coincide with the results obtained by (Camara et al., 2013) as they found *S. pyogenes* sensitive against these drugs. Vancomycin 100%, Chloramphenicol 100% and Tetracycline 67% showed complete sensitivity towards *S. pyogenes* (17 mm-23 mm) and these results are in agreement with the results of (Camara et al., 2013; Magiorakos et al., 2012). *S. pyogenes* strains were moderately sensitive to Clindamycin 33% and Azithromycin 33% and these results were also similar to (Camara et al., 2013) who found maximum sensitivity of these drugs against this specie. Highest sensitivity patterns were obtained against *S. bovis* specie as 83.33% strains were found sensitive against Streptomycin, Gentamycin, Chloramphenicol, Azithromycin and Ampicillin and along with this 66.67% strains were found sensitive against Oxacillin and Amoxicillin and above all 100% strains were sensitive against Vancomycin.

This result was obtained on the basis of sensitivity range being 15 mm or above. Results for Clindamycin and Tetracycline were different as 50% strains showed resistance against these drugs. The range of resistance was 15 mm and 18mm respectively. When antibiotic susceptibility of *E. faecalis* was concluded, Streptomycin along with Vancomycin, Chloramphenicol, Gentamycin and Azithromycin were found completely susceptible (15 mm-21 mm) to *E. faecalis* strains and this finding was in agreement with the results obtained by (Johnston et al., 2004). 87% strains were found sensitive to Streptomycin and Azithromycin whereas 100% strains were sensitive towards Vancomycin, Chloramphenicol and Gentamycin. *E. faecalis* was also found moderately sensitive against Ampicillin, Oxacillin Amoxicillin and Tetracycline where 50%, 40%, 62% and 50% strains were found sensitive respectively. This result was also in accordance with the findings of (Johnston and Jaykus 2004) Clindamycin was also sensitive against *E. faecalis* as 75% strains were found sensitive to this drug. This result was also similar to (Johnston and Jaykus 2004) who found 67% sensitive strains. No resistant strains were found in *E. faecalis*. There are many drug combinations available till date. In this study maximum eight combinations of drugs were tested against each *Streptococcus* species isolated. All of the isolates were tested against Gentamicin, Clindamycin, Amoxicillin, Ampicillin, Oxacillin, Chloramphenicol, Vancomycin, Azithromycin, Streptomycin and Tetracycline.

CONCLUSION

Infections caused by the *Streptococcus* species are with intense annoyance across the globe. The effectiveness of mainstream antibiotics is reducing gradually due to universal emergence of Multi-Drug Resistant (MDR) bacterial pathogens. The immediate modification of the conventional antibiotics along with the synthesis of new antibiotics is the basic requirement and the core issue which is prompted by the developing concerns regarding the antibiotic resistance. Modern compounds targeting the bacterial virulence can be synthesized to overcome the tremendous menace presented by multi-drug resistance MDR. Structural alterations of antibiotics can be achieved by development of influential structures from already existing antibiotics. This study has laid emphasis on the development of new classes of antibiotic or antimicrobial agents possessing various modes of action against MDR pathogens. In other words it can be stated that, to overcome the threats posed by the antibiotic resistance, antimicrobial compounds with a new mechanistic approach should be sought immediately.

REFERENCES

- Cole JN, Henningham A, Gillen CM, Ramachandran V, Walker MJ. (2008) Human pathogenic streptococcal proteomics and vaccine development. *Proteom Clin App* 2: 387-410. <https://doi.org/10.1002/prca.200780048>
- Miftahussurur M, Syam AF, Nusi IA, Makmun D, Waskito LA, et al. (2016) Surveillance of helicobacter pylori antibiotic susceptibility in Indonesia: Different resistance types among regions and with novel genetic mutations. *PloS one* 11: 166199. <https://doi.org/10.1371/journal.pone.0166199>
- Tettelin H, Nelson KE, Paulsen IT, Eisen JA, Read TD, et al. (2001) Complete genome sequence of a virulent isolate of *Streptococcus pneumoniae*. *Sci* 293: 498-506. <https://doi.org/10.1126/science.1061217>
- Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler VG. (2015) *Staphylococcus aureus* infections: epidemiology, pathophysiology, clinical manifestations, and management. *Clin Microbiol Rev* 28: 603-661. <https://doi.org/10.1128/CMR.00134-14>
- Ali Q, Khalil R, Nadeem M, Azhar MM, Hafeez MM, et al. (2020) Antibacterial, antioxidant activities and association among plant growth related traits of *Lepidium draba*. *Biol Clin Sci Res J* 2020: 11
- Danish P, Ali Q, Hafeez MM, Malik A (2020) Antifungal and antibacterial activity of aloe vera plant extract. *Biol Clin Sci Res J* 2020: 3.
- Garau G, Di-Guilmi AM, Hall BG. (2005) Structure-based phylogeny of the metallo- β -lactamases. *Antimicrob Agent Chemother* 49: 2778-2784. <https://doi.org/10.1128/AAC.49.7.2778-2784.2005>

Khalil R, Ali Q, Hafeez M.M, Malik A (2020) Phytochemical activities of *Conocarpus erectus*: An overview. *Biol Clin Sci Res J* 2020: 8.

Khalil R, Ali Q, Hafeez MM, Malik A (2020) Phenolic acid profiling by RP-HPLC: Evaluation of antibacterial and anticancer activities of *Conocarpus erectus* plant extracts. *Biol Clin Sci Res J* 2020: 10.

Levy SB, Marshall B (2004) Antibacterial resistance worldwide: Causes, challenges and responses. *Nat Med* 10: 122-129. <https://doi.org/10.1038/nm1145>

Fernández L and Hancock RE. (2012) Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev* 25: 661-681. <https://doi.org/10.1128/CMR.00043-12>

Mushtaq U, Mushtaq S, Afzal M, Ali Q, Malik A. (2020) Role of modern technology for treatment of HCV. *Biol Clin Sci Res J* 2020: 1.

Mika M, Mack I, Korten I, Qi W, Aebi S, et al. (2015) Dynamics of the nasal microbiota in infancy: A prospective cohort study. *J Allergy Clin Immunol* 135: 905-912. <https://doi.org/10.1016/j.jaci.2014.12.1909>

Rasmussen TT, Kirkeby LP, Poulsen K, Reinholdt J, Kilian M. (2000) Resident aerobic microbiota of the adult human nasal cavity. *Apmis* 108: 663-675. <https://doi.org/10.1034/j.1600-0463.2000.d01-13>

Von Eiff CK, Becker K, Machka K, Stammer H, Peters G. (2001) Nasal carriage as a source of *Staphylococcus aureus* bacteremia. *New England J Med* 344: 11-16. <https://doi.org/10.1056/NEJM200101043440102>

Walsh C. (2000) Molecular mechanisms that confer antibacterial drug resistance. *Nat* 406: 775-781. <https://doi.org/10.1038/35021219>

Magiorakos APA, Srinivasan, Carey RB, Carmeli Y, et al. (2012) Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18: 268-281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>

Martineau F, Picard FJ, Ke D, Paradis S, et al. (2001) Development of a PCR assay for identification of staphylococci at genus and species levels. *J Clin Microbiol* 39: 2541-2547. <https://doi.org/10.1128/JCM.39.7.2541-2547.2001>

Reiner K. (2010) Catalase test protocol. *ASM Microbe Library* 20: 45.

Johnston LM and Jaykus LA. (2004) Antimicrobial resistance of *Enterococcus* species isolated from produce. *App Environ Microbiol* 70: 3133-3137. <https://doi.org/10.1128/aem.70.5.3133-3137.2004>

Camara M, Dieng A, Boye CS. (2013) Antibiotic susceptibility of *streptococcus pyogenes* isolated from respiratory tract infections in dakar, senegal. *Microbiol Insight* 6: 71-75. <https://doi.org/10.4137/MBI.S12996>

Bauer AW, Perry DM, Kirby WM. (1959) Single-disk antibiotic sensitivity testing of staphylococci: An analysis of technique and results. *AMA Arch Interl Med* 104: 208-216. <https://doi.org/10.1001/archinte.1959.00270080034004>

Abera B, Kibret M. (2014) Azithromycin, fluoroquinolone and chloramphenicol resistance of non-chlamydia conjunctival bacteria in rural community of Ethiopia. *Indian J Ophthalmol* 62: 236-239. <https://doi.org/10.4103/0301-4738.99974>

Hudzicki J. (2009) Kirby-Bauer disk diffusion susceptibility test protocol. *Optomol* 6: 24.

Sami AJ, Khalid M, Jabeen S, Khalil S, Sajjad G. (2018) Nasal colonization and antimicrobial susceptibility pattern of staphylococcus species among children in Lahore, Pakistan. *J Med Microbiol Infect Dis* 6: 91-98. <https://doi.org/10.29252/JoMMID.6.4.91>

Jans C, Meile L, Lacroix C, Stevens MJ. (2015) Genomics, evolution, and molecular epidemiology of the *Streptococcus Bovis*/*Streptococcus Equinus* Complex (SBSEC). *Infect Genet Evol* 33: 419-436. <https://doi.org/10.1016/j.meegid.2014.09.017>

Li XZ and Nikaido H. (2004) Efflux-mediated drug resistance in bacteria. *Drugs* 64: 159-204. <https://doi.org/10.2165/11317030-000000000-00000>

Susan MP, Joyce de Azavedo, Donald E. Low, Kelly S. MacDonald. (1999) Antimicrobial Agents and Chemotherapy. *Chemother* 43: 1505-1507. <https://doi.org/10.1128/AAC.43.6.1505>

Verhoeven PO, Gagnaire J, Botelho-Nevers E, Grattard F, Carricajo A. (2015) Detection and clinical relevance of *Staphylococcus aureus* nasal carriage: An update. *Exp Rev Antiinfective Ther* 12: 75-89. <https://doi.org/10.1586/14787210.2014.859985>