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## ORIGINAL ARTICLE

# Resistance of Bacterial Pathogen of Dental clinic against the Antibiotics

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**ABSTRACT**

*Antibiotics are important adjuncts in the treatment of infectious diseases, including periodontitis. The most severe criticisms to the indiscriminate use of these drugs are their side effects and, especially, the development of bacterial resistance. The knowledge of the biological mechanisms involved with the antibiotic usage would help the medical and dental communities to overcome these two problems. Therefore, the aim of this research to check oral bacterial strains resistance against 6commercially available drugse.g.Penicillin (10U), Cefazolin (30µg), Norfloxacin (10µg), Tetracyclin (30ug), Oxacillin (1µg), Gentamycin (30µg) through the disc diffusion method. 100 sawabs were collected randomly from three dental clinics in Sargodha (Syed JanoodUllah Dental clinic, Dental consultant Drfarooq and Azhar Dental Surgery) and transfer to the laboratory of the Biosciences Department, University of Lahore, Sargodha Campus, where they were incubated overnight at 37˚C in the shaking incubator, gram staining and several biochemical test was perform to identify the bacteria. Result showed that most of the isolated bacteria strains were Gram positive 52% and 48% Gram Negative. Among these 98% were sensitive to NOR followed by the CN 95% and 86% against P and OX, TE 83%, 64 % against KZ. Furthermore the biofilm producers were more resistant to the antibiotics as compare to non-biofilm producers.*
*Key words: Antibiotic Resistance, Dental Biofilm, Disc Diffusion Method, Oral Micro-Flora.*

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## INTRODUCTION

In current era use of Antibiotics is an important source in fight against pathogen, in almost every field of life from food production to human medicine, since with the introduction of first antibiotic in 1937 namely, the sulfonamides, but the development of specific mechanisms of resistance has plagued their therapeutic use. Penicillin was first discovered by Alexander Fleming in 1928, but many years later it was used as antibiotic. Later in 1940, several years before the introduction of penicillin as a therapeutic, a bacterial penicillinase was identified by two members of the penicillin discovery team [1]. Once the

antibiotic was used widely, resistant strains capable of inactivating the drug became prevalent, and synthetic studies were undertaken to modify penicillin chemically to prevent cleavage by penicillinases (β-lactamases). Interestingly, the identification of a bacterial penicillinase before the use of the antibiotic can now be appreciated in the light of recent findings that a large number of antibiotic r genes are components of natural microbial populations [2]. There are many reason behind the development of antibiotic resistance and one of the reason is biofilm and Biofilms provides not only protection against altered pH, osmolarity, nutrients scarcity, mechanical and shear forces [3-5] but also block the access of antimicrobial drug and host immune cells to the planktonic bacteria with in biofilm [6,7]. Therefore, biofilm matrix gives the additional resistance power to bacteria which makes them to not only tolerate harsh conditions but also create hurdles in the cure of chronic infections and increase the risk of health issues. Another reason of development of antibiotic resistance is to transfer resistant gene from resistant bacteria to non-resistant bacteria with horizontal gene transfer, through conjugation [8]. Bio-films provides the opportunity to such transfer and make compatible conditions for activity, as increased genetic competence, high cell density, and accumulation of genetic elements. Few studies suggested that conjugation has been shown more efficient in biofilms as compared to planktonic ones [9-11]. To assess the antibiotic resistance and susceptibility against the antibiotic and link of biofilm with resistance.

## MATERIAL AND METHODS

### Collection and identification of Samples:

The samples were taken from 100 patients attending dental clinic (Syed JanoodUllah Dental clinic, Dental consultant Drfarooq and Azhar Dental Surgery). Samples were collected with the help of swabs and inoculated in the sterile eppendorf containing 1 ml nutrient broth. The samples were taken to the Laboratory of Biological Sciences University of Lahore Sargodha campus where they were incubated overnight at 37˚C in the shaking incubator. The bacterial isolates were identified by Gram staining, Cell morphology and biochemical tests and the results were compared with that of the known species. (Table 1)

### Disc- Diffusion Test for antibiotic susceptibility,

The inoculums were prepared by growth method. Isolates from the pure and maintained cultures were inoculated and incubated into. Trypticase soy broth until desired turbidity reached. The turbidity was adjusted to match that of a 0.5 McFarland standard. All the inoculums were used within 30 min. of inoculums preparation. **Inoculation and incubation**: In inoculums suspension, a sterile cotton swab was dipped and for removing of the extra moisture it was pressed against the wall of the test tube. The swab was then spread over the entire surface of agar plate and was allowed to absorb excess surface moisture. Within 15 min of plate inoculation, disc of given potencies were circulated evenly on the surface with the aid of sterile forceps. The plates were inverted and incubated at 35˚C (within 15 min. after the disc application) for 18-24 hours [9-13].

**Interpretation of results**: All of the plate was observed after incubation. Zones of inhibition were recorded in millimeters according to the procedure published by the Clinical Laboratory Standard Institute (CLSI) (table 2) and the experiment was repeated thrice for concordant results. All the data were statistically analyzed.

### Biofilm detection for Antibiotic resistant strains

Multi drug resistance strains were subjected to the detection of the biofilm through the process of tissue culture plate method TCP which is considered as golden method for the detection of biofilm.

Table. 1.Interpretive standards for Disc Diffusion susceptibility testing.

| S No. | Antibiotics | Disc Code | Class of Antibiotics | Zone Diameters (mm) | | | Source |
|---|---|---|---|---|---|---|---|
| | | | | Sensitive | Intermediate | Resistant | |
| 1 | **Penicillin G** | P | Penicillins | ≥28 | 20-27 | ≤19 | Oxoid 2013 CLSIFDA |
| 2 | **Cefazolin** | KZ | Cephalosporin | ≥18 | 15-17 | ≤14 | Oxoid 2013 CLSIFDA |
| 3 | **Norfloxacin** | NOR | Fluoroquinolone | ≥17 | 13-16 | ≤12 | Oxoid 2013 CLSIFDA |
| 4 | **Tetracyclin** | TE | Tetracyclins | ≥15 | 12-14 | ≤11 | Oxoid 2013 CLSIFDA |
| 5 | **Oxacillin** | OX | Penicillins | ≥13 | 11-12 | ≤10 | Oxoid 2013 CLSIFDA |
| 6 | **Gentamycin** | CN | Aminoglycoside | ≥15 | 13-14 | ≤12 | Oxoid 2013 CLSIFDA |

 

## RESULTS

### Antibiotic resistance:

All 18 isolated bacterial (table 3) strains from 100 samples when tested against 6 antibiotics showed various sensitivity and resistivity patterns. Maximum sensitivity for bacterial strains was recorded againstNOR 98%.followed by the CN 95% and 86% against P and OX, TE 83%, 64 % against KZ and Likewise the isolated bacterial strains exhibited sensitivity against most of the selected antibiotics are as follows 98% sensitivity against NOR, 95% against GN, 91% against CN, 83% against TE,(figure 1) and statistical analysis is summarized in the table 3.

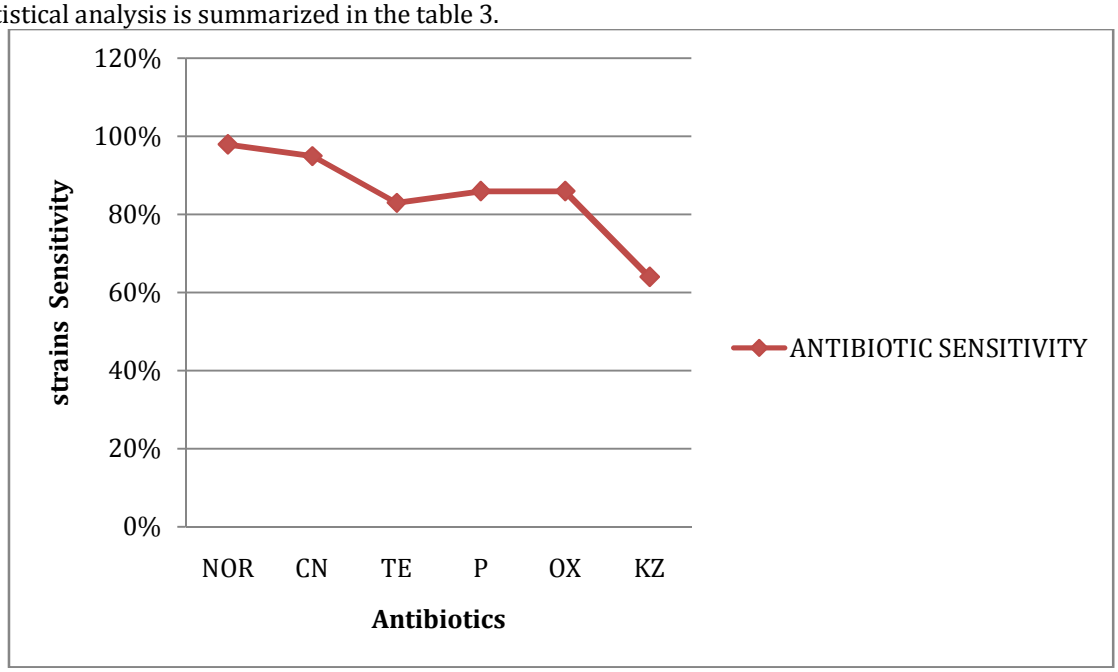


Figure 1: bacteria sensitivity against antibiotic.

### Evaluation of Antimicrobial susceptibility patterns (BF VS NBF)

When all level of biofilm forming (strong, moderate, weak / none) were analyzed for the antimicrobial resistance then it was indicated that Biofilm former (91%) higher antimicrobial resistance compared with non-biofilm formers (9%). Antimicrobial resistance pattern was observed for biofilm former and non biofilm former as follows (non-biofilm former vs. bio-filmformer) P [75{82.41%} VS 7 (77.77%)], KZ[65{70.65%} VS 7 (87.5%)], OX [80{87.91%} VS 8 (88.88%)] TE [85{70.1%} VS[12{80.2%}], CN [85{80.1%} VS[12{90.2%}], NOR [60{70.1%} VS[12{89.3.2%}] (figure 2).

Table. 2: Statistical Analysis for Antibiotic sensitivity

| Antibiotics | Mean zone inhibition (mm) | Std. Dev | Std. Error | *P* value |
|---|---|---|---|---|
| **Penicillin G** | 8.86 | 9.83 | 0.983 | 0.000 |
| **Cefazolin** | 14.79 | 12.08 | 1.208 | 0.000 |
| **Norfloxacin** | 32.2 | 6.473 | 0.6473 | 0.871 |
| **Tetracyclin** | 26.41 | 8.92 | 0.892 | 0.003 |
| **Oxacillin** | 3.15 | 5.72 | 0.572 | 0.426 |
| **Gentamycin** | 25.74 | 4.96 | 0.496 | 0.069 |

 

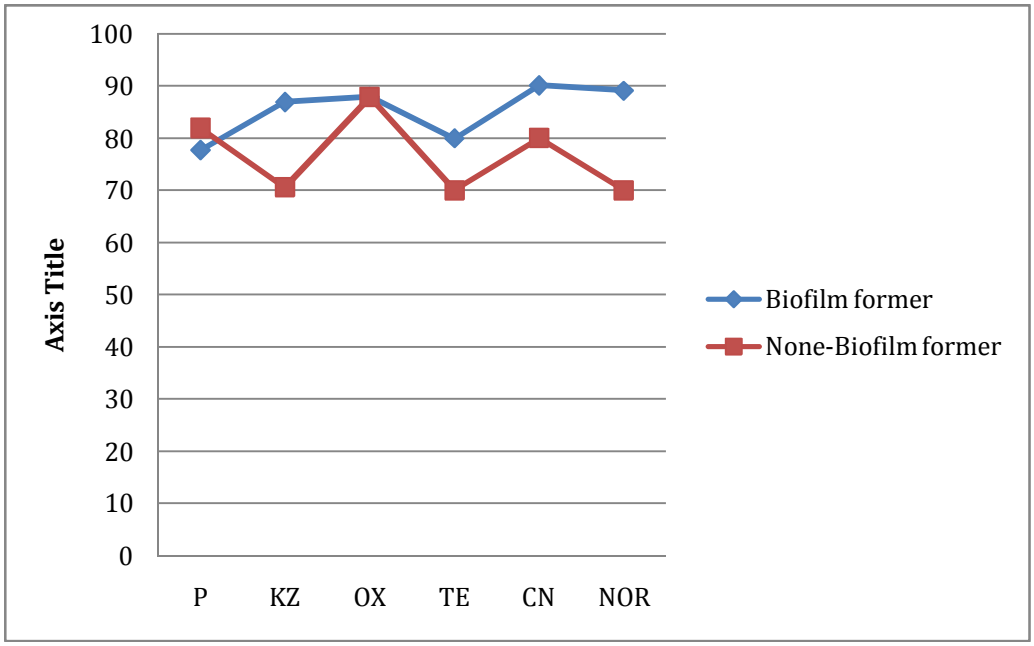


Figure 2: Comparison between biofilm former resistances vs. None-biofilm former

Table: 3: isolated bacterial strains from the dental clinics samples

| Gram positive Bacterial isolates | Frequency (%) | Gram Negative Bacterial isolates | Frequency (%) |
|---|---|---|---|
| *Streptococcus pneumonia* | 4(7.69) | *Acinetobacter radioresistens* | 6(12.5) |
| *Streptococcus pyogens* | 1(1.92) | *Klebsiella pneumonia* | 5(10.41) |
| Corynebacterium | 12(23.07) | *Escherschia coli* | 1(2.08) |
| Actinomyces 10 | 10(19.23) | *Y. pestis* 4 | 12(25) |
| *Micrococcus luteus* | 3(5.76) | *Neisseria spp* | 8(16.66) |
| *Clostridium difficile* | 6(11.53) | Veillonella | 12(25) |
| *S. sobrinus* | 2(3.84) | Haemophilus | 4(8.33) |
| *Corynebacterium spp* | 2(3.84) | Total | 48 |
| *Exiguobacterium spp* | 3(5.76) | | |
| Lactobacillus | 5(9.61) | | |
| *Bacillus cereus* | 4(7.69) | | |
| Total | 52 | | |

## DISCUSSION

Among 100 swabs 18 bacterial strains were isolated and identified as gram positive and gram negative through gram staining. In which the most re-dominant species were *Veillonella* (n= 12*)* followed by the *Neisseria spp* (n=8) in gram negative, on the other hand gram positive bacteria the most prevalent bacteria strain was *Clostridium difficile* (n=6) *followed by the* Lactobacillus(n=5) one of the oral cariogenic bacteria like *Klebisella,* were also identified in the present study. Each of the isolates is involved in the serious infections not only orally but also infects other sites of the body including skin, lungs, and eyes and can cause UTI. With the passage of time these microbial pathogen develops antimicrobial resistance result in the chorionic and delayed cured infection. Bacterial pathogens possess a number of ways to develop resistance including Mutation, HGT, and plasmid continuing gene of resistance and production of the Biofilm which actually create hurdles for the antibiotic penetration to reach the target. Due to drug abuse and long term usage of antibiotics, pathogens continuously develop antibiotic resistance even for the novel drugs [12]. Antibiotic administration should be on the base of the knowledge of the efficacy of antibiotic. It should be remembered that dental caries infections are such type of ecosystems in which by product of one bacterium may be nutrients for other species of bacteria [13].

Current studies reveal that Oxacilin and Norfloxacin could be the drug of choice in treating infections as very small amount of the isolates showed resistance to this antibiotic the only bacteria which shows resistance these were lactobaciilus, *E.coli* and Klebsiella. Norfloxacin is drug which can be orally absorbed with fluorine at position 6 and a piperazine ring at position 7. Specifically, [13]the antibacterial spectrum of norfloxacin includes *Pseudomonas aeruginosa*, as well as enteric pathogens. Norfloxacin is also active against both penicillin-susceptible and penicillin-resistant strains of *Neisseria gonorrhoeae*. Relative to its

 

activity against gram-negative bacteria, norfloxacin is somewhat less active against gram-positive cocci. In general, the *staphylococci* are more susceptible to the drug than are the *streptococci* [14] study conducted by Hussain Qadri and Steve Johnson in which out of the 151 isolates of gram-negative and gram positive bacteria were tested, 149 were inhibited by norfloxacin., concluded that norafloxcin could be a better option for many infection cure[15]. Many other studies also suggested that *E.coli* is actually norafloxcine resistance bacteria (consistent with current studies) and can favor many infections including prophylaxis[16].

But one thing which is more important matter to be concern is resistance shown by the isolated strains to the commonly prescribed antibiotics (gentamycin, pencilline and tetracyclin). Usually unnecessary and empirically administration of antibiotics by medical specialist gives rise to antibiotic resistant bacteria. Several studies were conducted by different researchers in different time concluded that community-associated MRSA were resistant(40%-100%) to penicillin, (34.78%-90%) to both tetracycline and gentamycin, norfloxacin (70%-81.4%), gentamicin (60%-73.13%) to tetracycline[17-19].

## CONCLUSION

Abuse of antibiotics hassled to many problems e.g. the appearance of Multi Drug Resistant bacteria which are not easy to control as these bacteria are resistant to most of the multi antibiotics. A drug policy should be planned by the hospitals and health institutions to control the pointless use of the antibiotics.

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