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ISOLATIONS AND CHARACTERIZATION OF ANTIBIOTIC RESISTANT BACTERIA FROM DENTAL PLAQUES

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ABSTRACT

- ***Isolate the pure endodontic bacterial colonies taking dental plaques as the sample***
- ***Characterize the organisms based on their antibiotic resistance.***

KEYWORDS: Isolations and Characterization of Antibiotic Resistant Bacteria

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Original Article

INTRODUCTION

Antibiotic resistance has been called one of the world's most pressing public health problems (1). Antibiotic resistance is not a new problem. Resistant disease strains began emerging not long after the discovery of antibiotics more than 50 years ago. Penicillin and other antibiotics, which were initially viewed as miracle drugs for their ability to cure such serious and often life-threatening diseases as bacterial meningitis, typhoid fever, and rheumatic fever, soon were challenged by some defiant strains (2). Antibiotic resistance is a major contributor to the disease, death, and costs resulting from hospital-acquired infections.

The ability of antibiotics to stop an infection depends on killing or halting the growth of harmful bacteria. Some bacteria have developed a natural resistance to antibiotics, long before the development of commercial antibiotics. If they are not naturally resistant, bacteria can become resistant to drugs in a number of ways. They may develop resistance to certain drugs spontaneously through mutation. Mutations are changes that occur in the genetic material, or DNA, of the bacteria. These changes allow the bacteria to fight or inactivate the antibiotic (6).

Bacteria also can acquire resistant genes through exchanging genes with other already resistant bacteria. The bacteria reproduce rapidly, allowing resistant traits to quickly spread to future generations of bacteria. This means that resistance can spread from one species of bacteria to other species, enabling them to develop resistance to multiple classes of antibiotics (6).

Procedure

Bacteria are collected from dental plaque and cultured in MS broth, MS agar and broth are selective for the organisms present in the plaque i.e., Streptococcus mutans, streptococcus mitis, streptococcus salivarius, and enterococcus sp and some bacilli species . Using MS agar and doing spread plate and streaking collect the colonies of individuals. Gram staining has been done for each individuals and their morphology was observed. Further screening for antibiotic resistance was carried on using antibiotics ampicillin, tetracycline, penicillin at different concentrations. Storage of the cultures will be done using the nutrient agar slants.

Isolation

The plaque sample is collected from the patient and then these sample organisms are spread on the M S agar plates which is a selective media for streptococci species. Then the individual colonies were picked and further to obtain pure colonies they were subjected to streaking. The pure colonies that were isolated included: bacilli(long rods and short rods) and coccobacilli. For the preservation purpose slants of nutrient agar were prepared.

Screening

The process of screening was done for checking antibiotic resistance among the isolated species. The antibiotics used included Ampicillin, Tetracycline, Penicillin at different concentrations as mentioned under 4.2.

Preparation of Media and Antibiotics

M.S.Agar (100ml)

Enzymatic Digest of Casein	1.5 g
Enzymatic Digest of Animal Tissue	0.5 g
Sucrose	5 g
Dextrose	0.1 g
Dipotassium Phosphate.	0.4 g
Trypan Blue	0.075 g
Crystal Violet	0.0008 g
Agar	1.5 g

Final pH: 7.0 ± 0.2 at 25°C

Nutrient agar (100 ml):

Nutrient broth powder	2.6 g
Agar	1.5g

Preparation of Antibiotic Pellets:

The antibiotics to be prepared include ampicillin, tetracycline, penicillin.

Ampicillin:

Stock……..500mg/ml

Working…….100µg/ml, 50µg/ml, 30µg/ml

Tetracycline

Stock……500mg/ml

Working……...50µg/ml, 30µg/ml

Penicillin

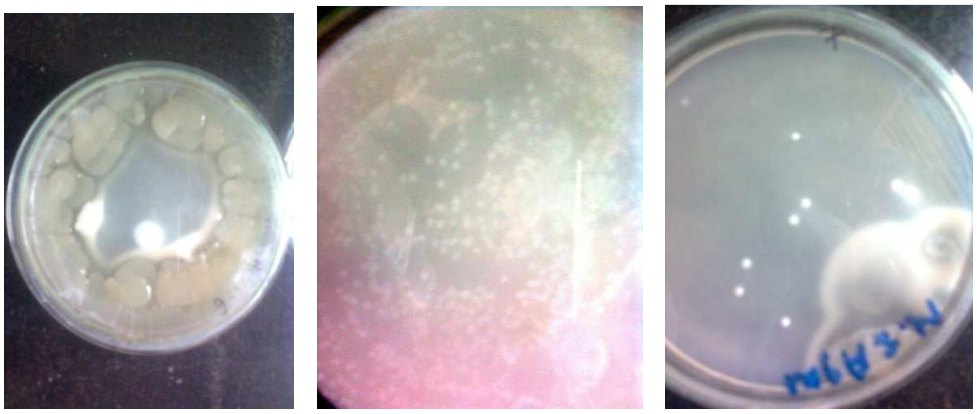
Stock…….800mg/ml

Working……400mg/ml, 200mg/ml

RESULT AND DISCUSSIONS

Results for Isolation

The pure colonies containing: coccobacilli sp. and bacilli sp..



Sample A(coccobacilli) Sample B(bacilli-long rods) Sample C(bacilli-short rods)

Figure 1

Results for Gram Staining

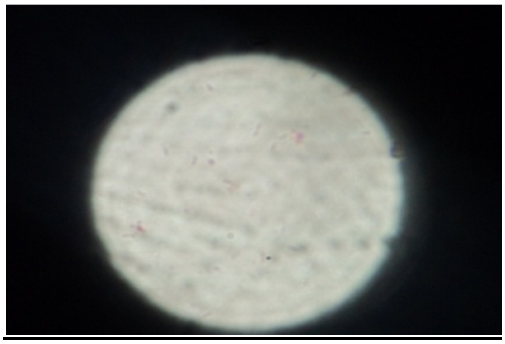


Figure 2: Coccobacilli (Gram Positive)

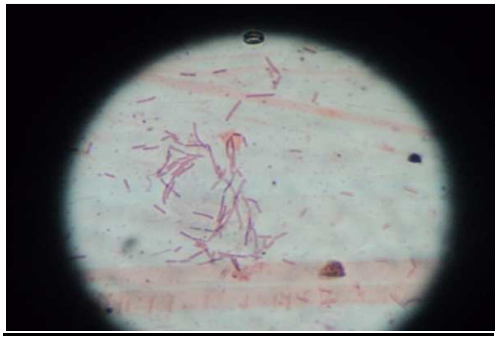


Figure 3: Bacilli (Long Rods)-Gram Negative

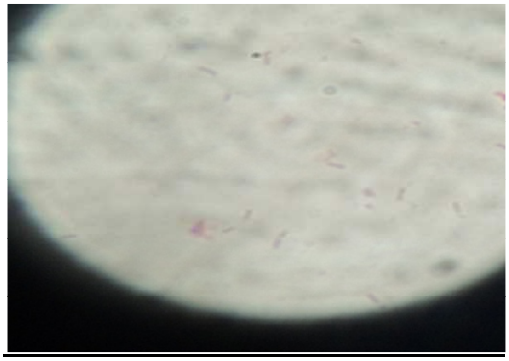


Figure 4: Bacilli (Short Rods)-Gram Negative

Results for Antibiotic Screening

The antibiotic resistance was shown partially by the bacilli(long rods),as well as the coccobacilli sp. for penicillin at 200mg/ml and 400mg/ml.

Figure 5

The above plates have the bacilli sp.(sample A) and coccobacilli sp.(sample B) which have shown this partial resistance.

Whereas the same organisms show partial resistance towards the antibiotic ampicillin at 30µg/ml concentration, but any of them did not show resistance towards other concentrations of ampicillin.

Figure 6

These bacteria did not exhibit any resistance towards any concentrations of tetracycline.

Figure 7

Sample C shows no resistance to any of the antibiotics,

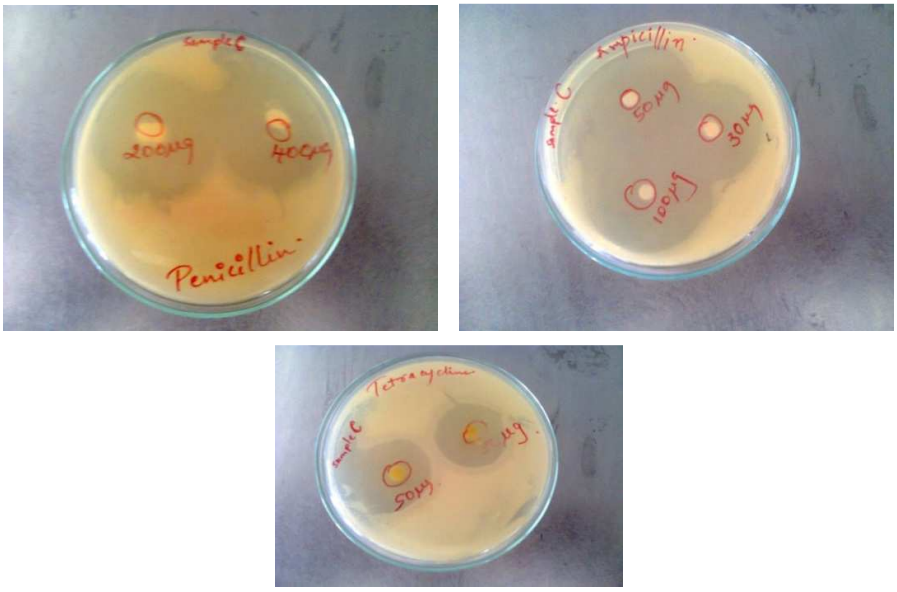


Figure 7

DISCUSSIONS

The antibiotic screening objective was successful. The organisms isolated from the dental plaques did show partial resistance towards the antibiotics as demonstrated above in the result. Thus required objective is met.

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