Prevalence of *Pseudomonas* in dental unit water of private dental clinics and its antimicrobial susceptibility using MAR index

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ABSTRACT

The bacteriology of water in a dental unit is important as potentially pathogenic microorganisms like *Pseudomonas* may be present in dental water unit, which could be fatal for immuno-compromised patients and be of significant morbidity in immuno-competent patients. It is difficult to treat infections caused by *Pseudomonas* due to their resistance to many commonly used disinfectants and antibiotics. The aim of the present study was to evaluate the *Pseudomonas* contamination in dental unit water lines of a dental hospital outpatient department and to analyse the antimicrobial susceptibility of *Pseudomonas*.

Keywords: *Pseudomonas*, dental, pathology, microbiology

INTRODUCTION

Microorganisms exist primarily by attaching to and growing upon living and inanimate surfaces resulting in biofilm formation. Biofilm formation is a process by which microorganisms irreversibly attach to and grow on a surface and produce extracellular polymeric substance (EPS) that facilitate attachment and matrix formation, resulting in an alteration in the phenotype of the organisms with respect to growth rate and gene transcription (1). Biofilms are seen in oral soft tissues and teeth, middle ear, gastrointestinal tract, urogenital tract, prosthetic heart valves, hip and joint prosthetic replacements, catheters, intrauterine devices, contact lens and dental unit water lines (2).

Dental unit waterlines are an integral part of the dental chair, supplying water as a coolant for air-turbines and ultrasonic scalar and are susceptible to biofilm formation. The bacteriology of water in a dental unit is important as potentially pathogenic microorganisms may be present in dental unit water which could be fatal for immuno-com-

promised patients and be of significant morbidity in immuno-competent patients (4). The contamination is also of considerable importance to dental professionals. The wide range of microorganisms includes gram positive bacteria such as Streptococcus, Staphylococcus, Mycobacterium and Actinomyces; gram negative bacteria such as *Pseudomonas*, *Escherichia coli*, *Klebsiella* and *Legionella*; yeast particularly, *Candida*; protozoa such as *Acanthamoeba*, *Cryptosporidium* and viruses particularly hepatitis B surface antigen (3,4,23).

It has been reported that most of the bacteria isolated from dental unit water lines are gram negative, heterotrophic bacteria. *Pseudomonas* is the most prevalent among the gram negative bacteria isolated from dental unit water and shown to cause nosocomial infection (14,15,19). *Pseudomonas* are aerobic, gram negative, motile bacilli. It is difficult to treat infections caused by *Pseudomonas* due to their resistance to many commonly used disinfectants and antibiotics (5,6). High prevalence of multiple antibiotic resistance indicates a serious need

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for surveillance and planning of effective interventions in such pathogens. In 1983, Krumperman described the Multiple Antibiotic Resistance (MAR) index as a good tool for health risk assessment. It is calculated as the ratio of number of antibiotics to which test isolate is resistant to total number of antibiotics to which the test isolate is exposed. MAR index values greater than 0.2 indicate high risk source of contamination where antibiotics are often used (7,8).

AIM

The aim of our study was to find the prevalence of pseudomonas in dental unit water. The objective of the study was to evaluate the presence of Pseudomonas contamination of dental unit water and to analyse the antimicrobial susceptibility of pseudomonas isolated from dental unit water using MAR index.

MATERIALS AND METHODS

This cross sectional study was conducted in ten private dental clinics. Water samples were collected from 10 dental clinics in different clinical specialities. Inclusion criteria included dental units that were in daily use for approximately a year.

Collection of samples

All water samples were collected from the air/water syringe (3 way syringe) using sterile technique. The sterile technique includes use of sterile gloves, wiping the external surface of air/ water syringe with 70% alcohol to prevent contamination and collection of sample in a sterile container. Water samples were collected in the mid-day and

the lines were flushed for 20 seconds after which 20 ml water was collected. Water splashing was minimised when filling the container and any contact between the air/ water syringe and the container was avoided. The water samples were labelled containing details of dental unit, department, date and time.

Transport of water samples

All the water samples in sterile containers were immediately transferred to the laboratory and processed for microbial evaluation.

Culture of water samples

Each water sample was centrifuged and 0.1 ml of the sediment was taken and then inoculated in Petri plates using streak method on the following culture media – nutrient agar (basal medium), MacConkey agar (differentiating medium), Blood agar (enriched medium) and Cetrimide agar (selective medium) (High-Media Laboratories). It was incubated at 37°C for 24 hours.

Isolation and identification

The plates were observed for growth. The isolated pathogens were identified for the presence of Pseudomonas on the basis of colony morphology, pigmentation, Gram staining and biochemical characteristics. The colony morphology that was noted for the presence of Pseudomonas were pearlescent appearance, grape like odour and yellow green pigmentation on cetrimide agar. Gram smear was done and gram negative rods were noted (Figure 1). Biochemical tests such as oxidase tests and catalase tests were done for confirmation (5).

FIGURE 1. Colony morphology in various agar. A – Nutrient agar; B – MacConkey agar; C – Blood agar; D – Cetrimide agar

Antibiotic sensitivity tests

All the confirmed *Pseudomonas* isolates were subsequently tested for antibiotic sensitivity patterns. Lawn culture was prepared on Mueller Hinton agar. The antibiotic sensitivity pattern was tested using agar diffusion (Kirby Bauer disc diffusion) method using nine different antibiotics discs (Clairo® Combi GN1) (Table 1). The antibiotics used were specifically anti-pseudomonal: Norfloxacin, Aztreonam, Cefotaxime, Gentamycin, Amikacin, Ciprofloxacin, Ofloxacin, Ceftazidime and Cefdinir.

TABLE 1. Antibiotics used in the study

ANTIBIOTICS	CONCENTRATION (mcg)
Norfloxacin	10
Aztreonam	30
Cefotaxime	30
Gentamycin	10
Amikacin	30
Ciprofloxacin	5
Ofloxacin	5
Ceftazidime	30
Cefdinir	5

Zone of inhibition was measured in millimetre (mm) using HiAntibiotic Zone scale (HiMedia Laboratory) to define sensitivity or resistance to different antibiotics. The risk assessment was done using Multiple Antibiotic Resistance (MAR) index determination for each isolate as described by Krumperman (1983). MAR index is calculated using the following formula (7):

MAR index = Number of antibiotics to which test isolate depicted resistance/total number of antibiotics used

RESULTS

Isolation and identification

Based on colony morphology observed on culture media, gram staining and biochemical tests, *Pseudomonas* was identified and isolated (Table 2). A total of 10 dental unit water samples were collected and assessed for *Pseudomonas* contamination. Out of these, 8 water samples (80%) were positive for *Pseudomonas*, one sample (10%) had no significant growth and one sample (10%) was suggestive of *E coli*.

Antibiotic susceptibility by *Pseudomonas*

Out of eight, seven isolates (87.5%) of *Pseudomonas* showed susceptibility to Norfloxacin fol-

lowed by Amikacin (six isolates). Out of eight, seven isolates (87%) of *Pseudomonas* showed high level of resistance to Ciprofloxacin and Cefdinir followed by Aztreonam, Ofloxacin and Ceftazidime (six isolates; 85.7%) (Table 3).

TABLE 3. Antibiotic susceptibility of *Pseudomonas* isolates from 8 samples

ANTIBIOTICS	Resistant isolates	Sensitive isolates
Norfloxacin	1	7
Aztreonam	6	2
Cefotaxime	4	4
Gentamycin	4	4
Amikacin	2	6
Ciprofloxacin	7	1
Ofloxacin	6	2
Ceftazidime	6	2
Cefdinir	7	1

Risk assessment using MAR index

The multiple antibiotic resistance indices were higher than the 0.2 limit in 7 out of 8 isolates (87.5%), indicating the high risk source of contamination in dental unit water (Table 4 and figure 2).

FIGURE 2. Antibiotic susceptibility of *Pseudomonas* isolates from 8 samples

TABLE 4. Antibiotic resistance profile and multiple antibiotic resistance (MAR) index of *Pseudomonas* isolates of each sample

Sample	Resistant isolate	Sensitive isolate	MAR Index	Inference (< 0.2)
1	7	2	0.7	High risk
2	6	3	0.6	High risk
3	-	-	-	-
4	6	3	0.6	High risk
5	6	3	0.6	High risk
6	9	0	1	High risk
7	4	5	0.4	High risk
8	5	4	0.5	High risk
9	-	-	-	-
10	0	9	0	Low risk

TABLE 2. Isolation and identification of *Pseudomonas* in each sample

Sample	Nutrient agar	Gram Stain	MacConkey agar	Blood agar	Oxidase test	Resistant isolate	Sensitive isolates	MAR index	Risk
1	small round yellowish green colonies	G- rods	NON-LACTOSE	Mucoid colonies with β hemolysis	+	7	2	0.7	HIGH RISK
2	small round yellowish green colonies	G- rods	NON-LACTOSE	Gray colonies with β hemolysis	+	6	3	0.6	HIGH RISK
3	Large, opaque, irregular round colonies with butyrous consistency and earthy odour with yellowish green pigment	G- rods	NO SIGNIFICANT GROWTH	No significant growth	No significant growth	-	-	-	
4	Large, opaque, irregular round colonies with butyrous consistency and earthy odour with yellowish green pigment	G- rods	NON-LACTOSE	Gray with metallic sheen, spreading colonies with β hemolysis	+	6	3	0.6	HIGH RISK
5	Large, opaque, irregular round colonies with butyrous consistency and earthy odour with yellowish green pigment	G- rods	NON-LACTOSE	Gray with metallic sheen, spreading colonies with β hemolysis	+	6	3	0.6	HIGH RISK
6	Small round mucoid colonies with distinct earthy odour	G- rods	NON-LACTOSE	Gray with metallic sheen, spreading colonies with β hemolysis	+	9	0	1	HIGH RISK
7	Small round mucoid colonies with distinct earthy odour	G- rods	NON-LACTOSE	Gray with metallic sheen, spreading colonies with β hemolysis	+	4	5	0.4	HIGH RISK
8	Small round mucoid colonies with distinct earthy odour	G- rods	NON-LACTOSE	Large metallic sheen colonies with undulate margin and beta-hemolysis	+	5	4	0.5	HIGH RISK
9	smooth greyish white colonies	G- rods	LACTOSE	Gray colonies with β hemolysis	-	-	-	-	
10	Large, opaque, irregular round colonies with butyrous consistency and earthy odour with yellowish pigment	G- rods	NON-LACTOSE	Gray colonies with β hemolysis	+	0	9	0	LOW RISK

DISCUSSION

The results of this study indicate that the water lines of dental unit remain a potential weakness site in control of infection in dental practice. Out of 10 water samples, *Pseudomonas* was isolated in 80% of the samples indicating the high risk source of contamination. The high level of *Pseudomonas* contamination demonstrated in this study confirmed the results of others (8-20). The high level of contamination in dental unit water could originate from two sources: the public water piped into the dental chair (due to narrow lumens and long periods of stagnant water) and suck back of patient's saliva into the line due to lack of anti-retraction valves. Microorganisms present in the oral cavity could be aspirated into the dental unit water lines using defective check valve (11,12).

In contrast, *Pseudomonas* was not isolated in a study conducted in Shimla. In this study, water samples were collected from air / water syringe of dental unit before and after flushing. *Staphylococcus* coagulase negative colonies were only demonstrated with no *pseudomonas* contamination. The climatic conditions in Shimla does not favour the *pseudomonas* growth in water reservoir (21). In another study, the authors investigated the microbial diversity of biofilms found in dental unit water systems by three methods: microscopic examination by scanning electron microscopy (SEM), acridine orange staining, and fluorescent in situ hybridization (FISH); second method, 55 cultivated biofilm isolates were identified by 16S ribosomal DNA (rDNA) sequencing; third method was a culture-independent direct amplification and sequencing of 16S subclones from community biofilm 16S rDNA. In this study, prevalence of *Pseudomonas* (5%) was comparatively lesser than other bacteria that includes *Leptospira* (20%), *Sphingomonas* (14%), *Bacillus* (7%), *Escherichia* (6%) (22).

In the present study, isolates of *Pseudomonas* showed susceptibility to Norfloxacin followed by Amikacin. This indicates that the drugs Norfloxacin and Amikacin can be used as drug of choice against *Pseudomonas* infection in dental treatment. However, Ciprofloxacin and Cefdinir which are highly resistant should not be used against infection. Norfloxacin (Fluoroquinolones) acts against *pseudomonas* by inhibiting the enzyme bacterial

DNA gyrase, which nicks double stranded DNA, introduces negative supercoils and then reseals the nicked ends. Amikacin (Aminoglycosides) binds to 30S ribosomes and inhibits protein synthesis. Ciprofloxacin acts similar to Norfloxacin but increased resistance to *pseudomonas* could be due to mutation in DNA gyrase that has reduced affinity for fluoroquinolone. Cefdinir (Third generation Cephalosporins) acts by inhibiting cell wall synthesis and resistance to cefdinir could be due to loss of permeability to the antibiotic (25).

The Multiple Antibiotic Resistance (MAR) indices were higher than 0.2 limit in 7 out of 8 isolates (Table 4) indicating high risk source of contamination and originate from an environment where many antibiotics are used. This increase in MAR index does not translate the actual contamination; nevertheless, appropriate measures need to be taken.

Various recommendations are put forward to reduce the microbial contamination: Flushing the water lines in between patients and at the beginning and end of the day is a simple and useful method to eliminate oral flora entering the waterlines via suck-back (19,20). Use of disinfectants such as sodium hypochlorite, hydrogen peroxide, Chlorhexidine to flush the dental unit waterlines is an effective method (23). A recent study concluded a newly available product, chlorine dioxide (ClO₂) as an effective disinfectant against biofilms of dental unit water lines tubing (24). Usage of Anti-retraction valves that are regularly monitored and maintained can be used to reduce contamination from the patient (13). All these systems require a strict adherence to maintenance protocols to perform to their full potential.

CONCLUSIONS

The result is important and should be considered based on the fact that the *Pseudomonas* is more prevalent in the water sample. Therefore, it is necessary to regularly monitor microbiological quality of water in dental unit water, including detection of opportunistic pathogens, prevent water stagnation and use various treatment procedures available to disinfect and reduce biofilm formation in dental unit water.

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