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CHARACTERIZATION OF BIOCIDES RESISTANT ISOLATES FROM DENTAL UNIT WATER LINE BIOFILMS BY CULTURE DEPENDENT APPROACH

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Abstract
The importance of biocides resistant bacterial strains in medicine, industry and the environment has gained significant attention. Microbial contamination of dental unit waterlines is thought to be the result of biofilm formation within the small-bore tubing used for these conduits. Our objectives were to characterize biocides resistant isolates from dental unit water line biofilm (DUWL) using the standard laboratory approaches. Growth curves of isolates established in biocides free and supplemented medium demonstrated less growth in the presence of biocides. O10 | P a g e ptimum pH was 7 whereas; optimum temperature was 37°C. Isolates showed resistance against multiple of heavy metals while fewer antibiotics. Genetic studies were accomplished by performing conjugation and transformation experiments. In two isolates (AWT 21 and PTNPF) transconjugants were observed, while no transformant was recorded in any case. Overall, the findings of this study can be used to profile the metabolic effects of new biocides or biocide combinations upon biocides resistant biofilm isolates from clinical environment.

Key words: Biocides, resistant isolates, dental unit water lines, characterization, heavy metals, antibiotics

Introduction

Bacterial infection remains one of the most serious complications associated with the use of indwelling medical devices. The clinical environment is particularly susceptible to contamination by bacterial pathogens that grow on surfaces in biofilms (1). The formation of surface biofilms can be regarded as a universal bacterial strategy for survival and for optimum positioning with regard to available nutrients. In addition, biofilm bacteria are substantially resistant to surfactants, biocides, and antibiotics (2). Two problems can arise from the presence of biofilms in a distributing aqueous

system. First, the biofilm can clog pipes and tubings or interfere with the proper function of mechanical devices. Second, bacterial populations living in this protected mode of growth produce planktonic cells that contaminate fluids and alter their properties or, in the case of pathogens, can result in food poisoning or infections (2). As a result, microbial biofilms constitute major industrial and

medical concerns. These concerns are now being realized in the dental profession.

Dental unit waterlines were coated with a well established biofilm made up of filamentous and bacillus-like microorganisms (1). It has long been known that the water collected at the output of dental unit waterlines (DUWL) is densely populated with microorganisms. This dense, planktonic, microbial population is now known to be a reflection of the colonization of the waterlines by biofilms. Water stagnation is thought to be in part responsible for the phenomenon (3). The bacteria isolated from the water in dental units belong to the community of water bacteria (3), but the finding that some potentially pathogenic microorganisms such as *Pseudomonas aeruginosa* (1) may be present in the water used to perform intraoral and sometimes invasive treatments has led to some concern in the dental community (3).

Attempts to control the DUWL contamination have included the use of filters and various biocides. However, bacteria in biofilm mode exhibit 3000 times more resistance than their planktonic counterparts (4). At the present time, commercially available options for improving dental unit water quality are limited and certainly involve additional cost. Among various methods to disinfect DUWL, one is the use of biocides (1).

Biocides are used extensively in healthcare settings for different applications: the sterilization of medical devices; the disinfection of surfaces and water; skin antisepsis; and the preservation of various formulations. In addition, there are now numerous commercialized products containing low concentrations of biocides, the use of which is controversial. Some professionals believe that the indiscriminate usage of biocides in the healthcare environment may not be justified and is detrimental in the long term, for example, by promoting the emergence of bacterial resistance to specific antimicrobials (2).

The activity of a biocide depends upon a number of factors, some inherent to the biocide, some to microorganisms. Among microorganisms most resistant to biocidal exposure are bacterial spores, followed by mycobacteria, Gram-negative, Gram-positive, and fungal microorganisms (2). Although there are exceptions within this summarized classification (e.g, some mycobacteria are relatively sensitive to disinfection), this attempt at distinguishing microorganisms according to their susceptibility to biocides gives useful information for the selection of an appropriate biocidal agent (4). However, it is not always possible to predict which microorganisms will be present on certain surfaces, although the organic load or the extent of microbial contamination, and the presence or not of a biofilm can be determined (2). An understanding of the characterization of resistant organisms is essential to ensure that a biocidal product/formulation is used properly.

The present investigation was designed to look at characterization of DUWL biocides resistant isolates from a dynamic standpoint through growth curve analysis and resistance against heavy metals as well as antibiotics, pH-temperature effect, and conjugation-transformation ability.

Materials and methods

Bacterial strains and growth conditions

Seven bacterial strains (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF and MWPNPC) were isolated from DUWL tubing samples of a priniciple dental unit Lahore, Pakistan following the method of Liaqat and Sabri (1). All strains were resistant to 100 µg ml^{-1} of eight biocides (5.25% NaOCl, 35% H_2O_2, 4% tween 20, 1% PI, 0.2% CHX, 1% EDTA and 1% phe) in L-agar. All strains were stored in microbank tubes (Pro-lab Diagnostics, Neston, Wirral, United Kingdom) at −80°C and were routinely grown in biocides (100 µg ml^{-1}) supplemented L- broth and on LB agar (Oxoid, Basingstoke, United Kingdom) at 37°C with shaking at 150 rpm for the planktonic cultures.

Morphological and biochemical characterization

Bacterial strains were characterized morphologically and biochemically following Gerhardt *et al*. (5).

Analysis of growth kinetics

Growth characteristics of all DUWL biofilm isolates were assessed at 37 or 42°C with shaking (150 rpm). Fifty microliters ($A_{1.00}$ at 600 nm) from an overnight culture of each strain was inoculated in biocides free and biocides supplemented (100µg ml^{-1} of all biocides) medium. Bacterial growth was assessed by measuring the optical densities at 600 nm of the samples with a Bioscreen C microplate reader (Labsystems, Finland) after different time intervals i.e., 0, 2, 4, 6, 8, 12, 18, 24, 30, 36, 42, and 48 hours.

Effect of pH and temperature on bacterial growth

The impact of pH and temperature on bacterial growth of DUWL biofilm was observed in biocides free and supplemented L-broth. Fresh inoculum from overnight cultures ($A_{1.00}$ at 600 nm) was given in all the flasks. Flasks were incubated at various pHs (6-9) and temperatures (20-42°C) with 150 rpm shaking.

Effect of Heavy metal and antibiotic

Different heavy metals and antibiotics were used to check the multiple metals and antibiotic resistance profile of the isolates. Antibiotics used included $NiSO_4$, $HgCl_2$, $ZnSO_4$, $Pb(NO_3)_2$, $MnSO_4$, K_2CrO_4, $CoCl_2$, $FeSO_4$ and $CuSO_4$, whereas heavy metals were TMP (Trimethoprim – 300 µg ml^{-1}), Cdx (Cephradoxil - 100 µg ml^{-1}), Cd (Cephradine 100µg ml^{-1}), Em (Erythromycin – 100 µg ml^{-1}), Tc (Tetracycline - 25 µg ml^{-1}), Cm (Chloramphenicol – 50 µg ml^{-1}), Dx (Doxycyclin - 25 µg ml^{-1}), Ap (Ampicillin - 2000 µg ml^{-1}), Km (Kanamycin - 100 µg ml^{-1}) and Sm (Streptomycin - 100 µg ml^{-1}).

Plasmid screening

For genetic analysis, bacteria were screened for the presence of plasmid by gel electrophoresis of total cell lysate method (6).

Conjugation and transformation

To characterize plasmid, conjugation and transformation experiments were performed.

For conjugation experiments, broth mating technique of Willets (7) was used. The recipients were *Escherichia coli* K12 strains DH5α (F- φ80dlacZΔM15 Δ(lacZYA-argF)U169 deoR, recA1 endA1 hsdR17(rk – mk + phoA supE44 λ- thi-1 gyrA96 relA1) and C600 (F^- tonA21 thi-1 thr-1 leuB6 lacY1 glnV44 rfbC1 fhuA1 λ^-). Transconjugants were scored at 37 °C by plating mixture on double selective plates i.e., plates containing 100 μgml^{-1} of all biocides, 300 μgml^{-1}ampicillin for C600 and 500 μgml^{-1} streptomycin for DH5α.

For transformation, plasmid DNA was extracted according to the method of Thomas (6). *E. coli* K12 strains DH5α and C600 (Birmingham university, UK) were made competent and transformed. Transformants were scored by plating 50 µl of each transformation mixture on biocide supplemented plates and incubated at 37°C for 24-48 hours.

Results

Characterization of biocides resistant DUWL isolates

Seven biocides resistant isolates (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF and MWPNPC), isolated by Liaqat and Sabri (1), were characterized morphologically, biochemically and physiologically. All strains were gram positive and rods except for AWT 33 (gram negative) and AWT 21 (cocci) (Table-1a). Except for AWT 21, all were aerobic and able to denitrify. All were able to hydrolyse starch and reduce nitrate to nitrite. None of them could hydrolyse arginine. These isolates exhibited good growth on blood agar without any hemolysis pattern (Table-1b).

TABLE 1A: SOME MORPHOLOGICAL CHARACTERISTICS OF BIOCIDE- RESISTANT ISOLATES.

CHARACTERISTICS	BATERIAL STRAINS						
	AWT 16a	AWT 21	AWT 28	AWT 33	PT 16	PTNPF	MWPNPC
Colony							
Visual colour	Light yellow	Off white	Light yellow	Off white	Off white	Off white	Off white
Form	Irregular	Irregular	Irregular	Irregular	Circular	Arborescent	Circular
Margin	Irregular	Irregular	Irregular	Irregular	Entire	Ramose	Irregular
Elevation	Raised	Flat	Flat	Flat	Convex	Flat	Raised
Colony size (mm)	0.4-0.5	0.4-0.5	0.4-0.5	0.2-0.3	0.4-0.5	0.2-0.3	0.1-0.3
Internal Characteristics	Smooth opaque	Smooth opaque	Coarsely granular	Smooth Opaque	Smooth Opaque	Smooth Opaque	Smooth transparent
Cell							
Motility	+	+	+	+	-	-	+
Cell shape	Bacilli	Cocci	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Cell Size (µm)	1.2-0.5	1-1	2.5-0.5	1.2-0.6	1.1-0.2	1.0-0.4	1.5-0.5
Gram staining	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Capsule staining	-	-	+	+	-	+	-
Spore staining	-	-	+	-	-	-	-

TABLE 1B: BIOCHEMICAL CHARACTERISTICS OF BIOCIDES -RESISTANT ISOLATES.

CHARACTERISTICS	BACTERIAL STRAINS						
	AWT 16a	AWT 21	AWT 28	AWT 33	PT 16	PTNPF	MWPNPC
Urease test	-	+	-	-	-	-	-
Catalase	+	+	+	+	+	+	+
Cytochrome oxidase	-	-	+	-	-	-	-
Oxidation fermentation	A	F.A	A	A	A	A	A
Nitrate reduction	+	+	+	+	+	+	+
Denitrification	-	+	-	-	-	-	-
Methyl red	-	-	-	-	-	-	-
Voges Proskauer	-	-	+	+	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+
Citrate utilization	-	+	-	-	-	-	-
Arginine hydrolysis	-	-	-	-	-	-	-
Triple sugar iron (slant/butt)	K/K	K/K	K/K	K/K	K/K	K/K	K/K
Growth on EMB	-	-	-	+	-	-	-
Growth on MacConkey	-	-	-	NLF	-	-	-
Growth on Blood agar	+	+	+	+	+	+	+

-, negative., +, positive., A, aerobic., F.A, facultative anaerobe., K, alkaline reaction; NLF, non lactose fermenting.

Growth Kinetic

The Gowth behaviour of all isolates showed better growth in biocides free medium than to biocides supplemented medium. 4-6 hours extended lag phase was observed in the presence of biocides, wheresas 2-4 hours lag phase was observed in biocides free medium. Log phase of growth started after 4, 6 and 8 hours in biocides free and supplemented It persisted for 36 (AWT 16a, AWT 21, AWT 28, AWT 33, PT 16, PTNPF, MWPNPC) hours in two in two media. Stationary phase started after 36 hours of incubation. It also commenced after 36 (AWT 21, AWT 33, MWPNPC), 42 (AWT 16a) and 48 (AWT 28, PT 16) hours in different strains. Afterwards death or decline phase was observed. Overall, it is evident from Figure-1 that after 24 hours isolates showed better growth in biocides free medium but after 48 hours growth was almost equal in biocides free and supplemented medium (Fig- 1a).

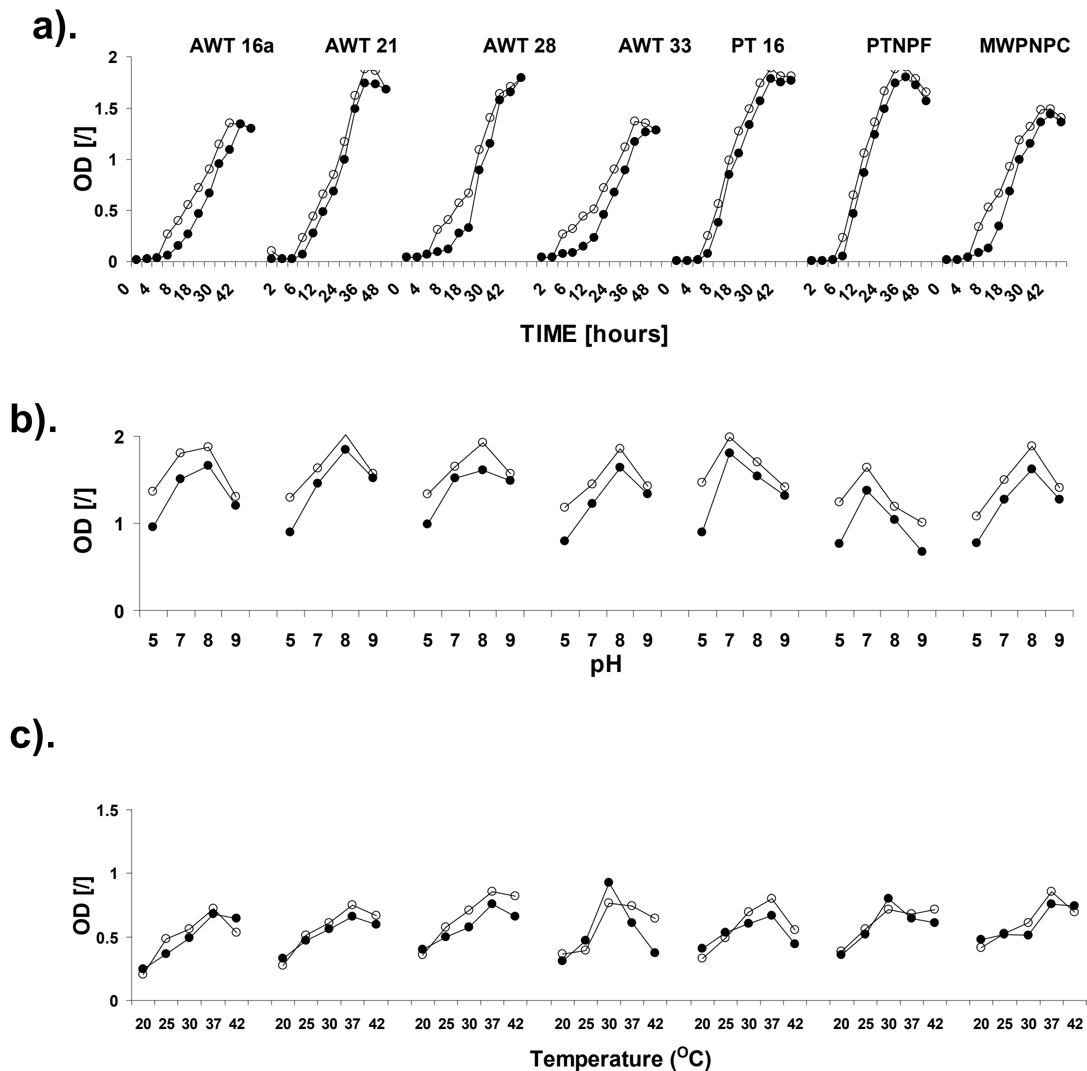

Fig. 1a. Growth curves of bioisolates in 100 µg biocides ml⁻¹ supplemented (●) and biocides free (○) L- broth; b). Growth of biocides resistant isolates a) at different pHs (5, 7, 8 and 9) and c). temperatures (20°C, 25°C, 30°C, 37 °C and 42°C) in biocides free (○) and 100 µg biocides ml⁻¹ supplemented (●) L- broth for 24 hours; Optical density was monitored at 600nm.

pH and temperature effect

Most of DUWL isolates preferred pH 8 for optimum growth except for PT isolates (PT 16 and PTNPF), which preferred pH 7. However, pH 5 has more detrimental effect on growth, particularly in biocides supplemented medium. Maximum growth was observed in biocides free medium compared to biocides supplemented medium. Likewise better growth was observed at 37°C and in biocides free medium in almost all isolates. However two isolates, AWT 33 and PTNPF exhibited maximum growth at

30°C. Almost equal growth was observed in AWT 16a and PTNPF in biocides free and supplemented media (Fig-1b-c).

Heavy metals and antibiotic resistance

All isolates were resistant against $NiSO_4$, $ZnSO_4$, $MnSO_4$, K_2CrO_4, $CoCl_2$, and $CuSO_4$, whereas all were sensitive to $Pb(NO_3)_2$ and $HgCl_2$ (Table-2). Antibiotic resistance profile of different isolates indicated that isolates were sensitive to majority of antibiotics including cephradine, erythromycin,tetracycline, doxycyclin, ampicillin and streptomycin. However they were resistant to trimethoprim and chloramphenicol (Table-3).

Determination of plasmid

All isolates were observed to harbour a plasmid. Plasmid residing in the biocides resistant isolates were designated as pBR6 (AWT 16a), Pbr7 (AWT 21), pBR9 (AWT 28), pBR10 (AWT 33), pBR13 (PT 16), pBR15 (PTNPF) and pBR19 (MWPNPC).

TABLE-2 HEAVY METAL RESISTANCE PROFILE OF BIOCIDES RESISTANT ISOLATES IN BIOCIDES FREE AND BIOCIDES SUPPLEMENTED (100MG ML^{-1}) L-BROTH.

BACTERIAL ISOLATES		HEAVY METALS (µg ml^{-1})								
		$NiSO_4$	$HgCl_2$	$ZnSO_4$	Pb $(NO_3)_2$	$MnSO_4$	K_2CrO_4	$CoCl_2$	$FeSO_4$	$CuSO_4$
AWT16a	0µg ml^{-1}	200	-	200	-	1000	700	5000	-	100
	100µg ml^{-1}	200	-	200	-	1000	700	5000	-	100
AWT 21	0µg ml^{-1}	200	-	200	-	1000	300	5000	200	100
	100µg ml^{-1}	200	-	200	-	1000	400	5000	200	100
AWT 28	0µg ml^{-1}	300	50	200	-	1000	700	5000	-	100
	100µg ml^{-1}	300	-	200	-	1000	700	5000	-	100
AWT 33	0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
	100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
PT16	0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
	100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
PTNPF	0µg ml^{-1}	300	-	200	-	900	700	5000	200	100
	100µg ml^{-1}	300	-	200	-	900	700	5000	200	100
MWPNPC	0µg ml^{-1}	300	-	200	-	1000	700	5000	200	100
	100µg ml^{-1}	300	-	200	-	1000	700	5000	200	100

- sensitive.

Conjugation and transformation

To identify the genetic determinant of biocides resistance in these strains, conjugation and transformation experiments were performed. *E. coli* strains DH5α and C600 were used as

recipients applying broth mating technique. Transconjugants in each case were selected on media supplemented with biocides (100^{-1}µg /ml)

TABLE-3 ANTIBIOTICS RESISTANCE PROFILE OF BIOCIDES RESISTANT ISOLATES IN BIOCIDES FREE AND BIOCIDES SUPPLEMENTED (100MG ML^{-1}) L-BROTH.

BACTERIAL ISOLATES		ANTIBIOTICS (µg ml^{-1})									
		TMP (300)	Cdx (100)	Cd (100)	Em (100)	Tc (25)	Cm (50)	Dx (25)	Ap (300)	Km (100)	Sm (500)
AWT16a	0µg ml¯1	+	-	-	-	-	+	-	-	+	-
	100µg ml¯1	+	-	-	-	-	+	-	-	+	-
AWT 21	0µg ml¯1	+	+	+	+	+w	+w	-	-	-	-
	100µg ml¯1	+	+	+	+	+w	+w	-	-	-	-
AWT 28	0µg ml¯1	+	-	-	-	-	+	-	-	+	-
	100µg ml¯1	+	-	-	-	-	+	-	-	+	-
AWT 33	0µg ml¯1	+	-	-	-	-	+	-	-	-	+
	100µg ml¯1	+	-	-	-	-	+	-	-	-	+
PT 16	0µg ml¯1	+	-	-	-	-	+	-	-	+	-
	100µg ml¯1	+	-	-	-	-	+	-	-	+	-
PTNPF	0µg ml¯1	+	+	-	+	+	+	-	-	+	+
	100µg ml¯1	+	+	-	+	+	+	-	-	+	+
MWPNPC	0µg ml¯1	+	-	-	-	-	+	-	-	+	-
	100µg ml¯1	+	-	-	-	-	+	-	-	+	-

+, resistant; - sensitive; +w weak growth.

TABLE-4 RESULTS OF CONJUGATION EXPERIMENTS USING *E. COLI* STRAINS DH5A AND C600 AS RECIPIENTS.

CONJUGATION				TRANSFORMATION		
DONOR STRAINS	PLASMIDS	RECIPIENT STRAINS		DONOR PLASMIDS	RECIPIENT STRAINS	
		E. coli			*E. coli*	
		DH5α	C600		DH5α	C600
AWT 16a	pBR6	-	-	AWT 16a	-	-
AWT 21	pBR7	-	-	AWT 21	-	-
AWT28	pBR9	-	+	AWT28	-	-
AWT33	pBR10	-	-	AWT33	-	-
PT16	pBR13	-	-	PT16	-	-
PTNPF	pBR15	-	+	PTNPF	-	-
MWPNPC	pBR19	-	-	MWPNPC	-	-

+, Transconjugants/Transformants obtained; -, No Transconjugants/Transformants obtained

and antibiotics (300 μgml^{-1} ampicillin for C600 and 500 μgml^{-1} streptomycin for DH5α) for which donor isolates were sensitive and recipients were resistant. Only isolates AWT 28 and PTNPF yielded transconjugants (Ap^R +

Biocides^R), when *E. coli* strain C600 was used as recipient. While no transformant was recorded in this study (Table-4).

Discussion

Dental clinics have been described to be contaminated by large number of potentially harmful microorganisms. Historically, the vast majority of attempts made at enumerating and control microbes present in DUWLs (1, 8, 9).

Various biocides have been used to combat the pathogens, a potential source of infection to patients visiting dental clinics (9). Because biocides tend to act concurrently on multiple sites within the microorganism, resistance is often mediated by non-specific means. Efflux pumps have the potential to act on a range of chemically dissimilar compounds and have been implicated in both biocide- and antibiotic-resistant bacteria. Cell wall changes may also play a role in the observed cross-resistance between biocides and antibiotics, probably by reducing permeability (10). Microbial changes that result in resistance to biocides and antibiotics should therefore cause concern.

This study deals with the characterization of biocides resistant microorganisms isolated from DUWL tubing samples in Pakistan. The different bacteria isolated from the dental unit water were characterized with respect to morphological, biochemical, physiological and genetical aspects. Morphological differences were observed among various isolates. Most of the isolates were off white in color, with irregular colony and margin and have flat elevation. Majority of these were gram positive, non capsulated and non spore formers. As reported in another study (11), the morphological variants differed from the wild type in attachment, biofilm formation, and cell attachment properties. This ability of biofilm bacteria to show morphological colony variants might be a strategy to tolerate a wide variety of environmental conditions (12). Various authors reported the presence of gram positive/negative isolates in DUWL settings (1, 13).

Growth curve analysis of DUWL isolates revealed better growth in biocides free medium. Whereas, comparatively less growth was observed in the biocides supplemented medium. This might be due to the fact that biocides have inhibitory effect on the synthesis of both nucleic acids and proteins, thus affecting the endogenous respiration. In addition, biocides also interfere with the energy yielding and energy requiring processes of the isolates thus suppressing their growth (14). A long lag phase observed in strains growing in biocides supplemented medium could be explained by the fact that the strains require some acclimation period to counterfeit the harmful effect of biocides on their growth (15).

Though isolates exhibited wide pH and temperature range in both media (biocides free and supplemented), but optimum pH

and temperature were 7 and 37°C respectively. Hence, these strains can be classified as alkaliphilics. Alkaliphilic bacteria also have more amino acids and sugars in their cell walls, which are important in determining their pH tolerance in the medium (16). Less growth observed at pH 5 in biocides supplemented medium might be due to the enhanced activity of some biocides (phenolics) as reported previously by Russel, (17), hence inhibiting the growth of the isolates. On the basis of their temperature preference (30-37°C), these can be categorized as mesophilic i.e., bacteria having optimum growth temperature of 25-42°C. Poor growth observed at 24°C might be due to inhibition of enzymatic activity, resulting in reduced rates of enzymatic catalyzed reactions (18).

Biocides resistant isolates exhibited resistance against multiple of heavy metals [$NiSO_4$ (200-500 μg ml^{-1}), $MnSO_4$ (900-1000 μg ml^{-1}), $CoCl_2$ (5000 μg ml^{-1}), K_2CrO_4 (300-700 μg ml^{-1}), $ZnSO_4$ (200 μg ml^{-1}), $FeSO_4$ (200 μg ml^{-1}) and $CuSO_4$ (100 μg ml^{-1})] while few antibiotics (trimethoprim and chloramphenicol). Studies regarding tolerance as well as susceptibility of biocide resistant bacteria to different antibiotics/heavy metals have been well documented previously (19, 20, 21) It has been claimed that widespread biocides use in hospital, domiciliary, industrial, and other settings contributes to the overall rate of drug resistance (22).

Many plasmids are self-transmissible and those which can be integrated in the host chromosome can also direct the conjugal transfer of chromosomal genes. Total cell lysate method indicated the presence of single plasmid in all the isolates. Conjugation and transformation experiments were performed to characterize the plasmid in biocides resistance isolates from dental settings. Only in two isolates (AWT 28 and PTNPF), transconjugants were scored whilst in none of the case, transformant was recovered on biocides supplemented plates. In one study, Gupta and Ali (23) monitored the transferable ability of mercury resistance plasmid from the wild type mercury resistance *E. coli* strains isolated from aquatic environments of India to the mercury-sensitive, naladixic acid-resistant recipient strain of *E. coli* K12 F_ lac_ by conjugation. Recipient strains were tested for the acquisition of mercury resistance L-agar plates containing different concentrations of mercury to which the donor strains were resistant and naladixic acid (30 μg ml^{-1}) to counter select against the recipient. Transconjugants obtained were able to tolerate the same concentration (25 to 55 μg ml^{-1}) of $HgCl_2$ as the wild-type strains.

For transformation, Gupta and Ali, (23) isolated plasmids from the wild-type *E. coli* strains and transferred into *E. coli* K12 strain DH5α by transformation using standard $CaCl_2$ procedure. Transformants were selected on L-agar plates

supplemented with different concentrations of $HgCl_2$ to which the donor strains were resistant. Transformants were obtained in each case on plates supplemented with different concentrations of $HgCl_2$. For rest of bacteria negative results were obtained. The unsuccessful results reflect that there might be involved some physiochemical condition, some additional requirement of nutrients or it might be that plasmid DNA is degraded by membrane bound nucleases of the competent (recipient) strains (24). Another possible reason might be that plasmid DNA be taken up by the cell but failed to replicate there or plasmid borne markers might not be expressed in the new environment (25).

In conclusion, the scientific community must weight the risk and benefits of using biocides in DUWLs. At present insufficient scientific evidence exist to weigh these biocides and additional precautions are needed to guide biocides development and use to inhibit microbial resistance. Present study investigates the various characteristics of biocides resistant isolates from DUWL biofilm which may be helpful in determining the objective evaluation of antimicrobial and antibiofilm products against these strains.

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