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The Biochemical Response of Oral Microorganisms to Common Dental Medicaments in Jamaica

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Abstract: **The objective of this study was to isolate and identify the oral microorganisms implicated in oral infections in the population and also to evaluate and classify the effectiveness of common dental medicaments. The study tested the potency of the medicaments against oral microorganisms.**
Methods: Bacteriological investigation was done on (140) oral samples. The samples were cultured in aerobic and anaerobic conditions on non-selective and selective media. Standard procedures of bacterial culture and identification were applied. Nine medicaments were utilized to test for sensitivity levels against isolates from the oral cavity.
Results: 12 microbes were identified. Some were pathogenic while others were opportunistic organisms. The highest sensitivity was demonstrated by *Acinetobacter calcoaceticus* and *Proteus mirabilis*. The lowest sensitivity was seen in *Pseudomonas aeruginosa* and *Hafnia alvei*. The most effective medicament against the isolates was Formocresol and the least effective medicament was Calcium hydroxide. Bacterial Survival Index test was performed to show the sum of colony formation units. The lowest survival rate was found in Formocresol dilutions while the highest survival rate was found in Calcium Hydroxide dilutions indicative of sensitivity and resistance respectively. Restriction enzyme analysis showed varying banding patterns among the DNA of the organisms. Unique bands were seen with varying sizes of 8,000 base pairs to 23,000 base pairs.
Conclusion: The oral microorganisms may have implications on the health of individuals harboring them. Therefore, the knowledge of medicaments and microorganism that inhabit the oral cavity is important in predicting and preventing not only dental diseases but also associated systemic complications.

Keywords: **Oral infections, microbes, medicaments, biofilm.**

1. INTRODUCTION

Understanding the characteristics of vast amount of microorganisms co-inhabiting the human body has increased drastically in recent years due to advances in biochemical and genomic techniques [17]. Different body regions including the skin, the mouth, vaginal tract and gastrointestinal tract contain distinct microbial communities [1]. The diversity of these microorganisms play a role in oral health and diseases [1]. The oral cavity contains a number of habitats in which varying microbial life exists, for example; teeth surfaces, saliva, tongue, hard and soft palate and sub gingival region [2]. Bacteria form an important group of microorganisms found in both healthy and diseased mouths [14]. There have been more than 300 types of bacteria found in the mouth [14]. Commensal bacteria are regarded as beneficial by defending against the colonization of invading pathogens [10].

Bacteria in the mouth are an issue everyone has to deal with some of the bacteria can be helpful. However, most of the bacteria are harmful and cause plaque and bad breathe [18]. There are toothpastes and other remedies that help to kill and prevent bacteria in people's mouth. Calcium carbonate and silicic acid ensure thorough removal of plaque. Regular brushing of the teeth removes bacterial plaque, which is mainly responsible for caries, paradentosis and tartar. With sea salt and minerals, in addition to extracts of amina, myrrh and yarrow, toothpastes ensure a healthy bacterial flora in the mouth. The pH value (7.0) of the toothpaste neutralizes acid which damage the teeth and may attack dental enamel. The presence of mucosa folds, interdental species, gums and other places where food, designated epithelium, and saliva are easily trapped creating favorable conditions for the reproduction of most micro-organism.

Many different products are currently marketed that promised to provide consumers with fresh breath. It is estimated that more than one billion dollars are spent annually worldwide on lozenges, chewing gum, mouth rinse, toothpaste and dentifrices in an effort to resolve this condition [18]. The active agents that are incorporated into treatment forms include surfactants, antibacterial agents, baking soda, peroxide; metal sacks herbal and natural extracts and chlorine dioxide [18]. Contaminated toothbrushes can also be a source for oral bacterial growth. Toothbrushes which are used regularly become contaminated with microorganisms that colonize the teeth and the oral cavity.

Some researchers believe that the oral cavity is a relatively easy environment for bacteria to colonize. This illustrates the unique ecology of the oral cavity and the specialized nature of the bacteria that reside in it [14]. Bacterial accumulation on oral surfaces is a major factor in the development of most of the common dental diseases such as dental caries and periodontal disease [18].

In this study, biochemical techniques were used to help in characterizing the pathogenic microbes from the oral cavity. Medicaments were utilized to test their potency against the microbes from the oral flora. The effects of the medicaments were examined using the isolates from the oral flora.

2. METHODS

Collection of Samples:

A total of one hundred and forty (140) oral samples were collected randomly in sterile bottles from participants. The participants were patients attending dental clinic in Linstead and Spanish Town St. Catherine Parish in Jamaica.

Isolation of Oral flora:

Each sample was streaked on the surface of: Blood, MacConkey and Chocolate agar media respectively. The isolates were then identified using their colony morphology, gram staining technique and biochemical analysis.

Antimicrobial Susceptibility:

Antibiotic test was done using disk diffusion (Kirby-Bauer) method [14]. Antibiotic disks Bacitracin(30µg), Optochin(50µg), Rifampin(30µg) Pencillin(30µg), Amoxicillin(30µg) Ceftazidime(30µg), Sulfamethoxidole(30µg) and Gentamicin(30µg) were placed on the inoculated plates and incubated at 37°C for 24 hours. After this period of incubation, the diameters of inhibition zones were noted and measured by a ruler in (mm), results were determined [9].

Medicaments Sensitivity:

Medicaments: Hydrogen Peroxide, Corsidine, Formocresol, Ugenol, Trich Acetic Acid, Sodium Hyperchloride, Camphorated Parachlorophenol, Calcium hydroxide and Zinc oxide were tested for sensitivity against the isolates. Inoculated plates were incubated at 37°C for 24 hours for growth. After this period of incubation, the diameters of inhibition zones were noted and measured by a ruler in (mm), results were determined [9].

Bacterial Survival Index (BSI):

The isolates were sub-cultured in nutrient broth and incubated at 37°C for 24 hours. Serial dilution method was then done using different concentrations of the selected dental medicaments. This replicated the approach used in [17] study with some modification and maintained the viability of the biofilm by providing moisture and nutrients from the agar base below. Bacterial survival index of the isolates was then determined based on the number of colony formation unit of the cells that survived after the experiment.

Restriction Enzyme Analysis:

DNA of the isolates was extracted using the MagNa Pure LC DNA Isolation Kit III (Roche). The DNA was then digested using the EcoRI and HindIII restriction enzymes (Fischer Scientific) and analyzed according to [8].

3. RESULTS

Identification of Isolates:

The isolates from oral samples were identified based on their colony morphologies in the media. The growth showed different sizes, shapes and pigmentation of the identified isolates in the oral samples. Direct gram stain of samples was also done to assist in the identification process. The gram staining technique categorized the bacteria isolates into two groups: gram positive and gram negative. There was evidence of gram negative rods, gram positive cocci and gram positive coccoid organisms. Biochemical analysis identified twelve microorganisms: *Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Staphylococcus aures, Serratia mercesenes, Salmonella species, Acinetobacter calcoaceticus* and *Staphylococcus epidermidis.*

Antimicrobial Susceptibility:

The isolates *Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Serratia mercesenes,* and *Acinetobacter calcoaceticus* were sensitive to amoxillin, ceftazidime, sulfamethoxidole and gentamicin. *Staphylococcus saprophyticus and Salmonella species* were sensitive to ceftazidime, sulfamethoxidole and gentamicin. *Pseudomonas aeruginosa, Hafnia alvei* were sensitive to ceftazidime and gentamicin but resistant to sulfamethoxidole. *Streptococcus mutan, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Staphylococcus aures, Serratia mercesenes, Salmonella species, Acinetobacter calcoaceticus* and *Staphylococcus epidermidis* were resistant to rifampin and pencillin.

Medicaments Sensitivity:

It was evident from medicaments sensitivity test that all the isolates were highly sensitive to Formocresol, and Trich acetic acid. *Pseudomonas aeruginosa* and *Hafnia alvei* were highly resistant to Hydrogen peroxide, Camphorated Parachlorophenol and Ugenol respectively. *Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Pseudomonas aeruginosa,* and *Staphylococcus aures* were sensitive to Corsidine. *Staphylococcus saprophyticus, Serratia mercesenes, Salmonella species, Staphylococcus epidermidis,* and *Acinetobacter calcoaceticus* were resistant to Corsidine. *Staphylococcus saprophyticus, Staphylococcus aures, and Staphylococcus epidermidis* were sensitive to Sodium Hypochlorite. *Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Pseudomonas aeruginosa, Serratia mercesenes, Salmonella species,* and *Acinetobacter calcoaceticus* were resistant to Sodium Hypochlorite. *Streptococcus mutan, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Staphylococcus saprophyticus, Pseudomonas aeruginosa, Staphylococcus aures, Serratia mercesenes, Salmonella species, Acinetobacter calcoaceticus* and *Staphylococcus epidermidis* were all resistant to Calcium Hydroxide and Zinc Oxide.

Bacterial Survival Index (BSI):

Bacterial survival index showed the variations in BSI of the isolates. The data varied across amongst different concentrations of medicaments. It was evident from the results that the number of the cells that survived in each medicament varied when compared to the control. Formocresol had the least BSI of 47 survival cells and Calcium hydroxide had the highest BSI of 341 survival cells. The control had BSI 338 survival cells.

Restriction Enzyme Analysis:

Restriction enzyme analysis showed banding differences among the extracted genomic DNA of the organisms. Unique bands of DNA fragments were seen with different sizes of 8,000 base pairs to 23,000 base pairs.

4. DISCUSSION

This study provides insight into antimicrobial effects of different common dental medicaments investigated. Based on the results obtained in the study, twelve (12) oral microorganisms were identified. These organisms were predominantly identified in different age groups and they are implicated in diseases of the oral cavity. Organisms *Streptococcus mutans* and *Streptococcus mitis* were predominant between the age groups (10-15) mostly children and (15-20) teenagers.

These organisms are pathogenic in oral cavity. This could be as a result of not brushing properly after meals or lack of dental care in children.

Organisms: *Acinetobacter calcoaceticus, Staphylococcus aureus, Klebsiella oxytoca, Proteus mirabilis and Hafnia alvei,* were predominant at the older age groups of (70-80) and (80-90). This could be as a result of weak immune system developing in the human body as cells/tissues degenerate. Organisms: *Staphylococcus saprophyticus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Serratia mercescens* and *Salmonella sp.* occurred both in young age groups (30-40) and middle age groups (40-50). These were opportunistic pathogens. Therefore, the prevalence of these organisms was more age related as seen from the age group graph. Antibiotics Ceftazidime, Gentamicin, Amoxillin and Sulfamethoxazole/Trimethoprim (SXT) were effective respectively against all microorganisms isolated. This is because all the organisms identified were highly sensitive based on the high peak that was seen in the graph of antimicrobial sensitivity. These antibiotics proved to have antibacterial properties to treat oral infections and diseases that could be caused by the isolates. The greatest sensitivity to antimicrobial was demonstrated by *Acinetobacter calcoaceticus* and *Proteus mirabilis.* However, these organisms were also resistant to Pencillin and Rifampin. The size of the zone and the rate of antibiotic diffusion were used to estimate the bacteria's sensitivity to the antibiotics. Formocresol, Ugenol, Tric Acetic Acid, and Camphorated Parachlorophenol were effective respectively against the identified microorganisms due to their sensitivity to the medicaments.

The most effective medicament was Formocresol and this could have antibacterial properties that need to be considered in the treatment of oral infections and diseases. This is because all the twelve isolates were highly sensitive to Formocresol based on the highest peak shown in the Medicament graph. The least effective medicament was Calcium Hydroxide and it should be reduced or avoided in treatment of oral infections. This is because all the identified microorganisms were highly resistant to this medicament. Bacterial survival index was low in the organisms when utilized against Formocresol medicament. A high bacteria survival index was seen in Calcium Hydroxide. These results showed that organisms have defensive properties and mechanisms of resistance. Restriction enzyme analysis showed banding differences among the extracted genomic DNA of the organisms. The presence of the unique bands was an indication that the organisms may produce a gene that confirms the pathogenic effect of the organisms. This gene may cause resistance to the medicaments in the oral flora.

5. CONCLUSION

The findings from this Jamaican study showed that formocresol medicament was the most potent against microbes of oral flora. Formocresol had the highest antimicrobial effect compared to the other medicaments. It could be considered in the treatment of oral cavity infections and diseases. From the results in this study, formocresol consistently showed a higher sensitivity and a lower bacterial survival index (BSI) to all isolates. The medicament proved to be successful in treatment of oral cavity infections. However, this medicament should not be used unnecessary because of the possibility for the organisms to develop resistance. Preventive measures should be utilized in oral hygiene to avoid invasion of oral microorganisms. Due to genetic variability and adaptability over time antimicrobial susceptibility test should be done every five years to ensure that antimicrobial resistance is not developed by microbes. Thus, the knowledge of medicaments and microorganisms that inhabit the oral cavity is important in predicting/ preventing not only dental diseases but also associated systemic complications caused by them.

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APPENDIX - A

Tables and Figures:

	Table 1: Shows colony morphology and gram staining			
Sample	**Blood agar**	**Mac Conkey agar**	**Chocolate agar**	**Gram Staining**
102-C1	C1- small (glossy)	No growth	C2- small colonies	+ (cocci)
	Gray colonies			

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102-C2	C2- medium glossy		C2- medium glossy	+ (cocci)
111-C2	C2- small/grey colonies	No isolation	C2- small/grey colony	+ (cocci)
113-C1	C1- large glossy (W)	C1- large (LLF)	C1- large glossy	+ (cocci)
113-C2	C2- medium glossy	C2- medium- NLF	C2- medium glossy	
115-C1	C1- large gray	No isolation	No isolation	+ (cocci)
115-C2	C2- small gray			- (rods)
117-C1	C1- fine glossy (G)	No isolation	C2- fine glossy gray	- (rods)
117-C2	C2-large glossy		C1-large glossy	- (rods)
	Gamma			
119-C1	C1 -large glossy	No isolation	No isolations	- (rods)
119-C2	C2- fine glossy			- (rods)
120-C1	C1- large glossy	No isolation	C1- large glossy	+ (cocci)
120-C2	C2- fine		C2- fine	
	Gamma		Gamma	
121-C1	C1- large glossy		C1- large glossy	+ (cocci)
			C2- large glossy	+ (cocci)

Table 1 shows the growth of colonies in blood agar, MacConkey agar and Chocolate agar. The colony morphology showed different sizes, shapes and pigmentation of the isolates from the oral samples. There was evidence of gram negative rods and gram positive coccoid organisms.

Key:C1 - Large colonies, C2 - Small colonies, C3 - Fine colonies, LF - Lactose Fermentation NLF - Non Lactose Fermentation, W - White, G - Gray, + Positive, - Negative

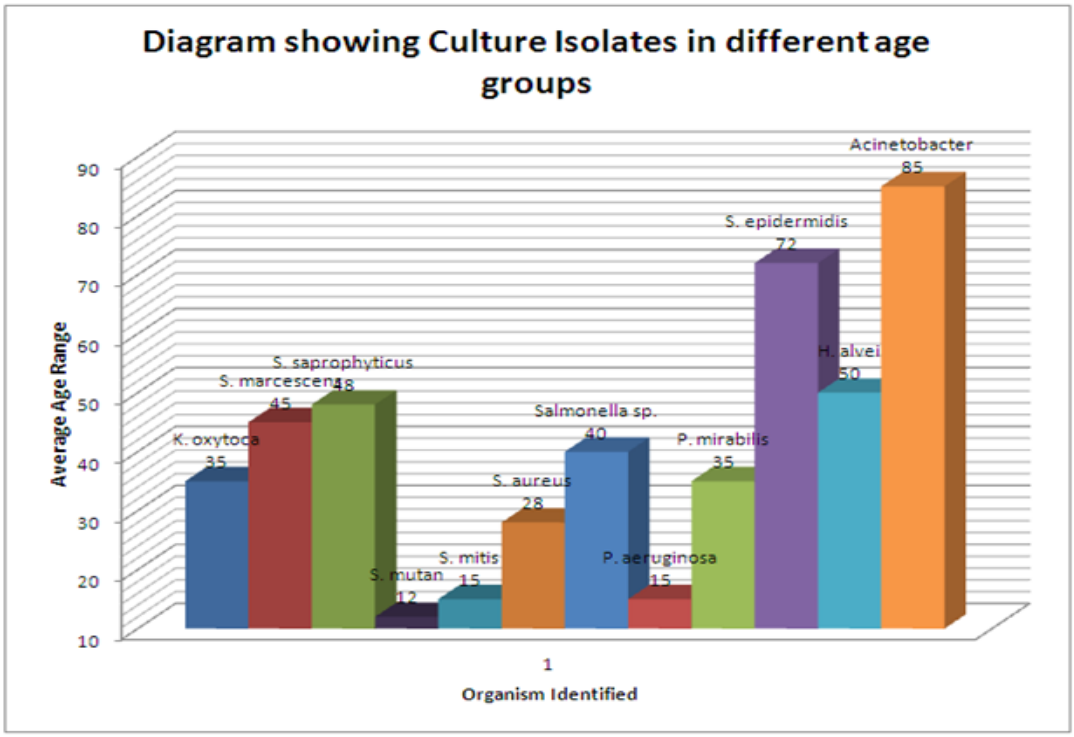

Figure 1

Figure 1 show how the twelve isolates identified from the oral samples was predominant in different age groups (10 yrs – 85 yrs). The analysis of oral samples via gram staining and other biochemical test identified twelve microorganisms: *Streptococcus mutans*, *Streptococcus mitis*, *Klebsiella oxytoca*, *Proteus mirabilis*, *Hafnia alvei*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Serratia mercesens*, *Salmonella species*, *Acinetobacter calcoaceticus* and *Staphylococcus epidermidis.*

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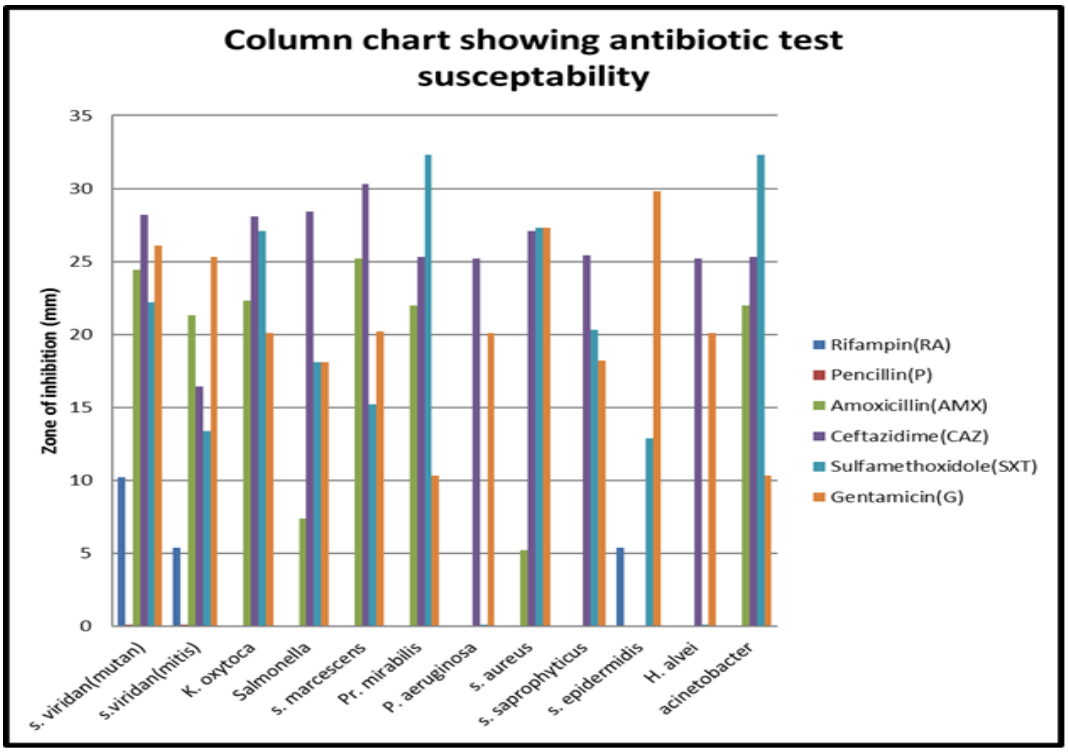

Figure 2

Figure 2 shows Acinetobacter calcoaceticus and Proteus mirabilis were highly sensitive to Sulfamethoxidole. Streptococcus mutan, Klebsiella oxytoca, Serratia mercesenes, Salmonella species, Pseudomonas aeruginosa, Staphylococcus aures, Staphylococcus saprophyticus, and Hafnia alvei were sensitive to ceftazidime. Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Salmonella species, Serratia mercesenes, Pseudomonas aeruginosa, Staphylococcus aures, Staphylococcus saprophyticus, Staphylococcus epidermidis, and Hafnia alvei were sensitive to gentamicin. Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Serratia mercesenes Proteus mirabilis and Acinetobacter calcoaceticus were also highly sensitive to amoxilin. However, Rifampin and pencillin had the lowest peak from the graph showing how all these organisms were resistant to the antibiotics.

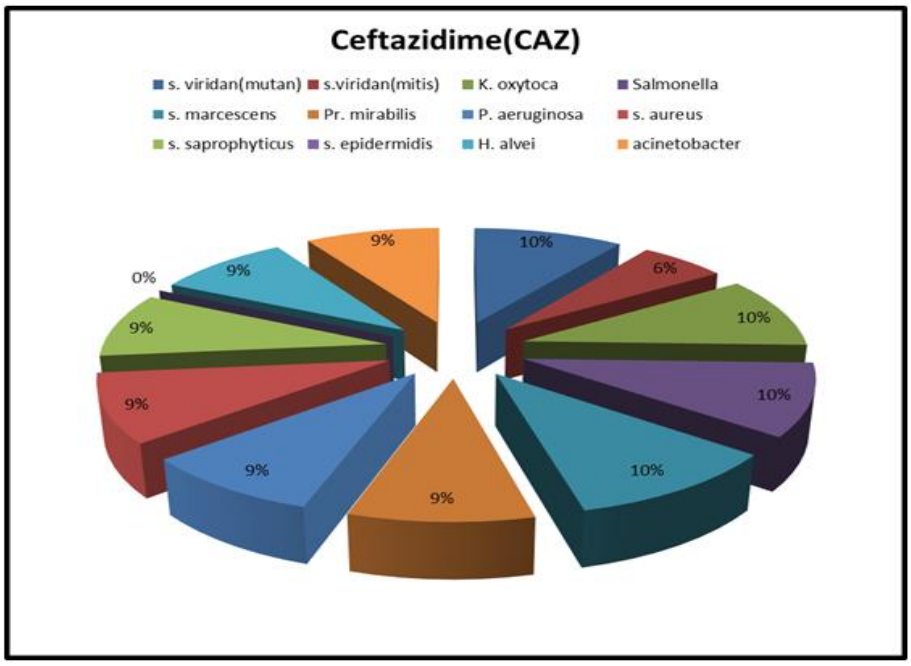

Figure 3

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Figure 3 shows a pie chart showing sensitivity percentages (%) of culture isolates to Ceftazidime antibiotic. The highest sensitivity was 10% and the lowest sensitivity was 0%.

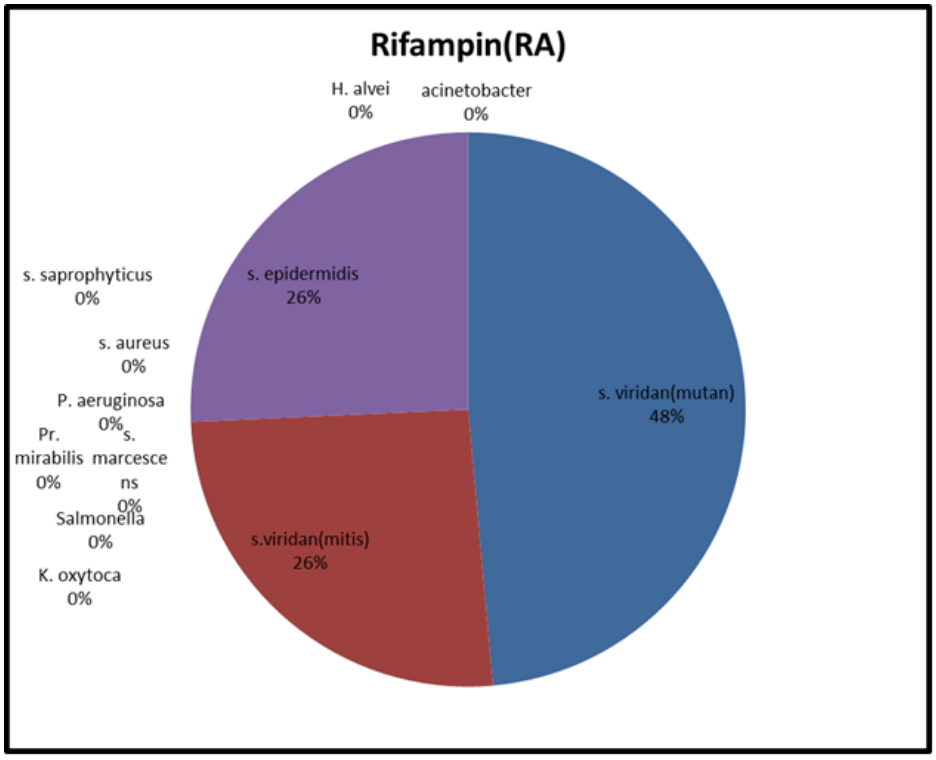

Figure 4

Figure 4 shows pie chart showing sensitivity percentages (%) of culture isolates to rifampin antibiotic. The highest sensitivity was 48% and the lowest sensitivity was 0%.

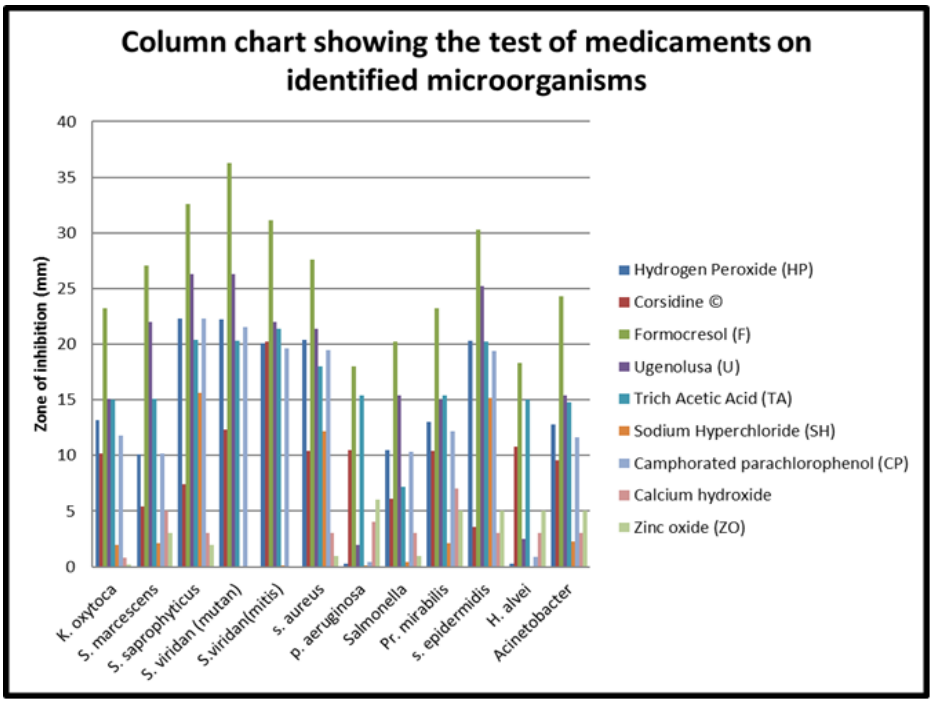

Figure 5

Figure 5 shows the sensitivity of isolates tested against selected common dental medicaments. *Streptococcus mutans, Streptococcus mitis, Klebsiella oxytoca, Proteus mirabilis, Hafnia alvei, Staphylococcus saprophyticus, Pseudomonas*

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aeruginosa, *Staphylococcus aures*, *Serratia mercesenes*, *Salmonella species*, *Acinetobacter calcoaceticus* and *Staphylococcus epidermidis* were *highly* sensitive to Formocresol. *Klebsiella oxytoca*, *Proteus mirabilis*, *Hafnia alvei*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Staphylococcus aures*, *Serratia mercesenes*, *Salmonella species*, *Acinetobacter calcoaceticus*, *Staphylococcus epidermidis*, *Streptococcus mutan and Streptococcus mitis* were also sensitive in Ugenolusa, Trich Acetic Acid, Hydrogen Peroxide and Corsidine respectively.

Staphylococcus saprophyticus, *Pseudomonas aeruginosa*, *Staphylococcus aures*, *Serratia mercesenes*, *Salmonella species*, *Acinetobacter calcoaceticus*, *Staphylococcus epidermidis*, *Streptococcus mutan*, *Streptococcus mitis*, *Klebsiella oxytoca*, *Proteus mirabilis*, and *Hafnia alvei* were highly resistant to Zinc Oxide, Sodium Hypochlorite and Calcium Hydroxide.

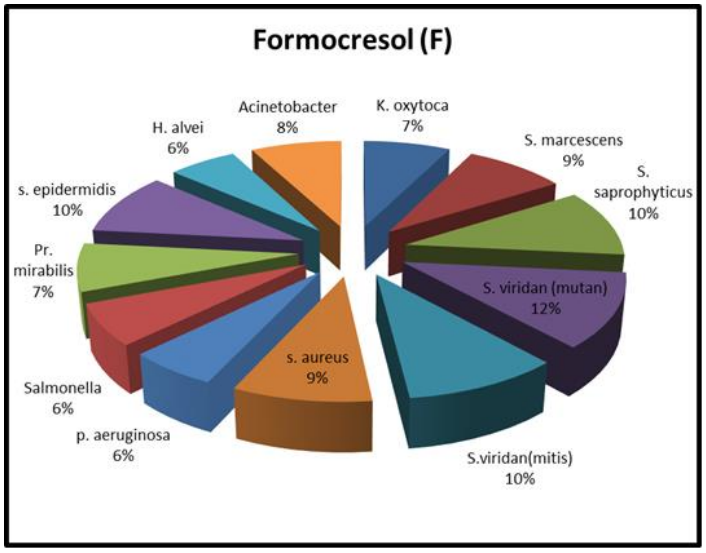

Figure 6

Figure 6 pie chart shows sensitivity percentages of culture isolates to Formocresol Medicament. The highest sensitivity was 12% and the lowest sensitivity was 6%.

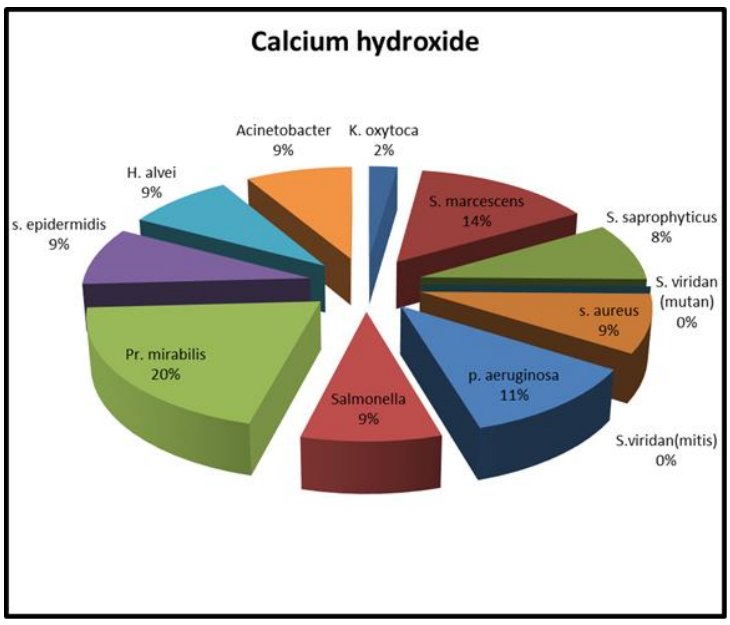

Figure 7

Figure 7 pie chart shows sensitivity percentages of culture isolates to Calcium Hydroxide Medicament. The highest sensitivity was 20% and the lowest sensitivity was 0%.

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Table 2 Bacterial Survival Index (BSI)

		Number of colony cells survived after serial dilution						
Isolates	**Medicament**	**10^{-1}**	**10^{-2}**	**10^{-3}**	**10^{-4}**	**10^{-5}**	**10^{-6}**	**Total survival cells**
	Hydrogen peroxide	136	17	9	4	0	0	166
	Corsidine	90	60	30	12	5	0	197
	Fomocresol	25	15	7	0	0	0	47
	Ugenol	32	28	16	0	0	0	76
	Trich acetic acid	135	50	32	21	10	2	250
	Camphorated Parachlorophenol	46	20	9	5	1	0	81
	Sodium hypochlorite	140	98	50	32	10	2	332
	Calcium hydroxide	139	95	60	25	17	5	341
	Zinc Oxide	138	80	61	30	16	3	328
	Control	144	100	48	27	15	4	338

Table 2 shows Bacterial Survival Index (BSI) average of the twelve isolates in response to the medicaments. The Medicaments and control were used to determine the survival of isolates on biofilm induced filter paper disc in the Muller Hinton agar plates. These data varied across amongst different concentrations of the medicaments. The highest BSI was Calcium hydroxide and the least BSI was Formocresol.

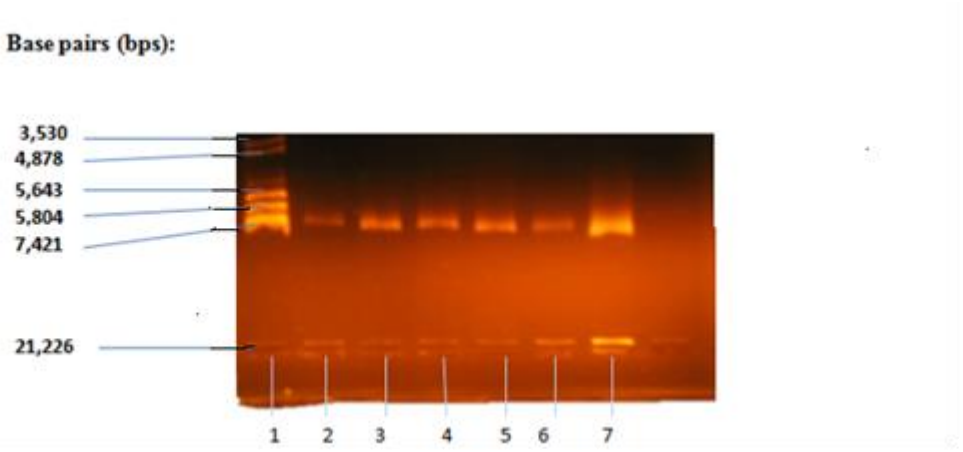

Figure 8

Figure 8 shows EcoR I restriction enzyme analysis of the isolates genomic DNA. In lane 1 is the molecular marker/DNA ladder. Lane 2 *Proteus mirabilis, lane 3 Hafnia alvei, lane 4 Streptococcus mitis, lane 5 Pseudomonas aeruginosa, lane 6 Staphylococcus aures* and *lane 7 Streptococcus mutans.*

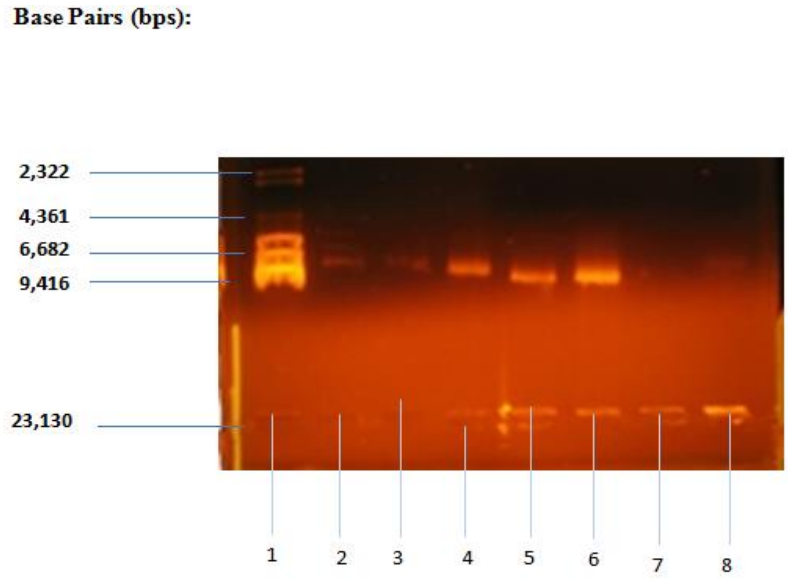

Figure 9

Figure 9 shows Hind III restriction enzyme analysis of the isolates genomic DNA. In lane 1 is the molecular marker/DNA ladder. Lane 2 *Proteus mirabilis, lane 3 Hafnia alvei, lane 4 Streptococcus mitis, lane 5 Pseudomonas aeruginosa, lane 6 Staphylococcus aures* and *lane 7 Streptococcus mutans.*