Original Article

Microbial Analysis and Determination of Antibiotic Susceptibility of Dental Laboratory Equipments and Laboratory Attire

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Abstract

Context: This study was done to determine the level and type of microbial contamination present on the surface of various dental laboratory equipment and laboratory attire and to determine the antibiotic susceptibility pattern of these isolated pathogens. **Subjects and Methods:** The samples were divided into following groups: six groups of dental laboratory equipment (articulators, facebow, fox plane, polishing buff, micromotor handpiece, and surveyors) and dental attire of laboratory technicians and students. A total of 33 swabs were collected from each dental laboratory equipment, namely, articulators, facebow, fox plane, polishing buff, micromotor handpiece, and surveyors. The dental laboratory attire of students and dental technicians were analyzed separately. The swabs were collected from the laboratory attire at the end of the week, and they were washed once a week and at the beginning of the week. The groups are Group 1 – dental laboratory attire (students), Group 2 – dental laboratory attire (technicians), Group 3 – polishing buff, Group 4 – facebow, Group 5 – surveyor, Group 6 – fox plane, Group 7 – articulator, and Group 8 – micromotor handpiece. The moistened swabs were inoculated into the broth and subcultured on to the MacConkey Agar plates, and then incubated aerobically at 37°C for 24 h. The organisms were identified based on colony morphology, Gram staining, and standard biochemical tests. The antibiotic susceptibility patterns of the isolated organisms were done according to the CLSI guidelines. The collected data were statistically analyzed. **Statistical Analysis Used:** The data collected were entered into a Microsoft Excel Spreadsheet and analyzed using IBM SPSS Statistics, Version 22 (Armonk, NY, IBM Corp.). The frequency and mean standard deviation of the samples were analyzed using Fisher's exact value test. Percentage of resistance among the isolates to different antimicrobials was also determined. **Results:** The microorganisms isolated were *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative *Staphylococcus*, *Pseudomonas*, *Klebsiella*, nonfermenting Gram-negative bacteria, and *Bacillus* species. The mean microbial levels in dental laboratory attire were more (5 $\log_{10}$ colony-forming units [CFU]) compared with dental equipment (3 $\log_{10}$ CFU–4 $\log_{10}$ CFU). Furthermore, most of the isolated organisms showed increased antimicrobial resistance. **Conclusion:** Majority of the isolated organisms were not a part of the normal oral microflora and are capable of causing various diseases. The increased resistance to the antimicrobials showed by the isolated organisms proves that there are increased chances of multiresistant organisms to occur in the future.

Keywords: *Cross infection, laboratory attire microbes, microbial analysis*

Introduction

The cross contamination in the dental clinics and dental laboratories is a growing concern nowadays after several studies found that the transmission of infection is mainly by the contaminated impressions or improper disinfection of the laboratory equipment or improper handling of the clinical items after arrival at the dental laboratories.[1] Certain bacteria which are not a part of normal flora can cause serious diseases if passed to patients whose prosthesis are made in the contaminated areas of dental laboratory and handled by persons with contaminated laboratory attire. Infection control programs should be developed and completed before handling any clinical items from the dental clinics.

The knowledge of microorganisms harboring the dental equipment and the laboratory attire is important in minimizing cross contamination and improve safety of both dental clinicians and laboratory technicians. The identification of microorganisms from the commonly used dental instruments and laboratory attire will help in the implementation of required sterilization and disinfection methods. This will also help in improving the patient safety by reducing the risk of nosocomial infections.

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Subjects and Methods

The study was conducted by collecting samples from the laboratory attire of the students, dental technicians, and dental equipment in A B Shetty Memorial Institute of Dental Sciences, Mangalore, with aid from Department of Microbiology, K.S. Hegde Medical Academy, Mangalore, for microbial analysis. The sample was 33 swabs each from dental equipment, namely, articulators, facebow, fox plane, polishing buff, micromotor handpiece, and surveyors. A total of 33 swabs each were collected from the laboratory attire of the students and dental technicians [Figure 1]. The swabs were collected from the laboratory attire at the end of the week and they were washed once a week and at the beginning of the week.

The samples were divided as follows: Group 1 – dental laboratory attire (students), Group 2 – dental laboratory attire (technicians), Group 3 – polishing buff, Group 4 – facebow, Group 5 – surveyor, Group 6 – fox plane, Group 7 – articulator, and Group 8 – micromotor handpiece. The sterile swabs were dipped in saline before the samples were collected. The site from which the samples were taken in the dental laboratory attire were pockets, as the microbial load was more in this region according to the other studies conducted [Figure 2].[2] The swabs were transported to the microbiology laboratory in sterile vile [Figure 3].

The moistened swabs were inoculated into the broth and subcultured onto MacConkey Agar plates. The plates were then incubated aerobically at 37°C for 24 h. The organisms were identified based on colony morphology, Gram staining, and standard biochemical tests [Figure 4].

The antibiotic susceptibility patterns of the isolated organisms were done according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The organisms were inoculated into the broth and the turbidity was adjusted to 0.5 McFarland concentration. With the sterile swab, the organisms were streaked on to the Muller–Hinton agar plates. The discs were placed into agar and the plates were incubated aerobically at 37°C for 24 h. The zones of inhibition were measured using a scale, and the susceptibility was assigned as per the CLSI guidelines.

Statistical analysis

The data collected were entered into a Microsoft Excel Spreadsheet and analyzed using IBM SPSS Statistics, Version 22 (Armonk, NY IBM Corp). The frequency and mean standard deviation of the samples were analyzed using Fisher's exact value test. Percentage of resistance among the isolates to different antimicrobials was also determined.

Results

Microbial analysis of the swabs taken from the dental laboratory attire of the students and technicians showed more microbial contamination than the swabs from the dental laboratory instruments. The microorganisms isolated from the dental laboratory attire of the students and technicians were *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative *Staphylococcus*, *Pseudomonas*, *Klebsiella*, nonfermenting Gram-negative bacteria,

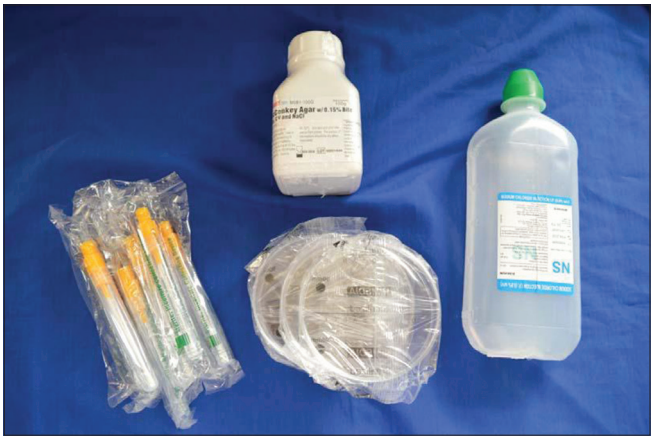

Figure 1: Armamentarium for the study

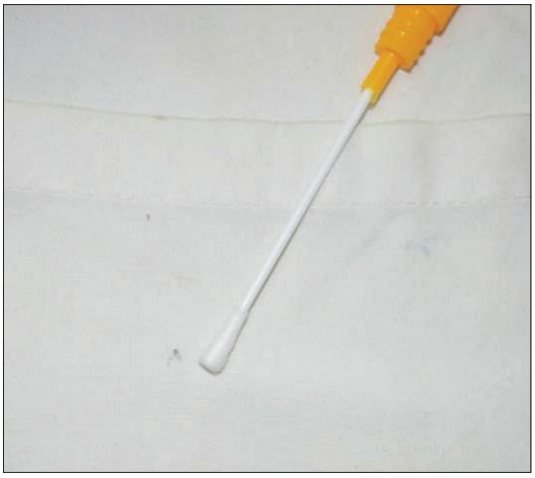

Figure 2: Sample collection

Figure 3: Transport of the sample in a sterile test tube

and *Bacillus* species. The swabs taken from the dental instruments also showed the same microorganisms as the dental laboratory attire, except coagulase-negative *Staphylococcus*.

The microbial levels were found to be almost similar among the samples from dental laboratory attire of the students and dental technicians. The mean level of microorganisms were 5 $\log_{10}$ colony-forming unit (CFU) of *S. aureus*, 5 $\log_{10}$ CFU of *E. coli*, 5 $\log_{10}$ CFU of *Pseudomonas*, 5 $\log_{10}$ CFU of *Klebsiella*, 5 $\log_{10}$ CFU of nonfermenting Gram-negative bacteria, and 5 $\log_{10}$ CFU of *Bacillus* species in the dental laboratory attire (Group 1 and Group 2). The level of microbes was comparatively less in the dental instruments than the dental laboratory attire. The level of microorganism in the polishing buff (Group 3) was 4 $\log_{10}$ CFU of *Pseudomonas*, 3 $\log_{10}$ CFU of *Klebsiella*, and 3.75 $\log_{10}$ CFU of *Bacillus* species. Facebow (Group 4) showed 4 $\log_{10}$ CFU of *Pseudomonas*, 3.5 $\log_{10}$ CFU of *Klebsiella,* and 3 $\log_{10}$ CFU of *Bacillus* species. Surveyors (Group 5) showed 4 $\log_{10}$ CFU of *Pseudomonas* and 3 $\log_{10}$ CFU of *Bacillus* species. 3 $\log_{10}$ CFU of *S. aureus*, 3 $\log_{10}$ CFU of *E. coli*, 4 $\log_{10}$ CFU of *Pseudomonas*, and 3 $\log_{10}$ CFU of *Bacillus* species were seen in fox plane (Group 6). 3 $\log_{10}$ CFU of *S.aureus*, 3 $\log_{10}$ CFU of *E. coli*, and 4 $\log_{10}$ CFU of *Pseudomonas* were seen in the samples from articulator (Group 7) [Figure 4]. Micromotor handpiece (Group 8) had 3 $\log_{10}$ CFU of *S. aureus*, 4 $\log_{10}$ CFU of *Pseudomonas*, 3 $\log_{10}$ CFU of *Klebsiella*, and 3 $\log_{10}$ CFU of nonfermenting Gram-negative bacteria [Table 1].

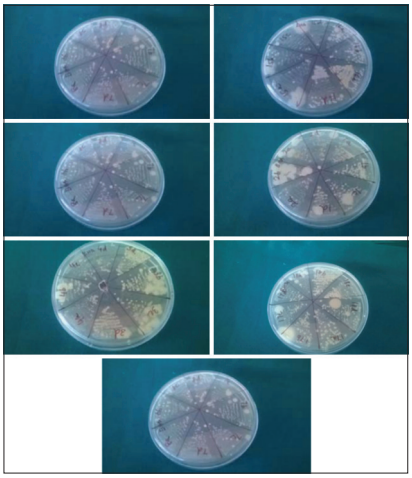

Figure 4: Colony-forming units of different pathogens seen as clusters in various groups[1-8]

The comparison of presence of microbes in the dental laboratory attire of students and dental technicians showed statistically significant difference in the presence of *Pseudomonas* species [Table 2a and b]. Where $p<0.05$ was considered significant.

The comparison of the presence of *Staphylococcus* among the different dental equipment were statistically significant (Fisher's exact value = 17.84, $P < 0.001$). The presence of nonfermenting Gram-negative bacteria had a Fisher exact value of 9.127 and was statistically significant ($P < 0.01$). The comparison of the presence

Table 1: Mean microbial levels among the positive samples in different group

Group	Mean $\log_{10}$ (CFU [SD])					
	Staphylococcus aureus	***Escherichia coli***	***Pseudomonas***	***Klebsiella***	**NF Gram-negative bacteria**	***Bacillus* species**
1	5.00 (0)	5.00 (0)	5.00 (0)	5.00 (0)	5.00 (0)	5.00 (0)
2	5.00 (0)	5.00 (0)	5.36 (0.5)	5.00 (0)	5.00 (0)	5.00 (0)
3	.	.	4.00 (0)	3.00 (0)	.	3.75 (0.5)
4	.	.	4.00 (0)	3.50 (0.58)	.	3.00 (0)
5	.	.	4.00 (0)	.	3.00 (0)	.
6	3.00 (0)	3.00 (0)	4.00 (0)	.	.	3.00 (0)
7	3.00 (0)	3.00 (0)	4.00 (0)	.	.	.
8	3.00 (0)	.	4.00 (0)	3.00 (0)	3.00 (0)	.

*The comparison of presence of microbes in the dental laboratory attire of students and dental technicians showed statistically significant difference in the presence of *Pseudomonas* species. CFU: Colony-forming units; SD: Standard deviation; NF: Nonfermenting

Table 2a: Comparison of presence of (*Staphylococcus aureus*, *Escherichia coli*, coagulase-negative *Staphylococcus*, and *Klebsiella pneumoniae*) in Group 1 and Group 2

Group	***Staphylococcus aureus***		***Escherichia coli***		**Coagulase-negative *Staphylococcus***		***Pseudomonas***		***Klebsiella pneumoniae***	
	Absent	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**	**Absent**	**Present**
1, *n* (%)	26 (78.8)	7 (21.2)	27 (81.8)	6 (18.2)	29 (87.9)	4 (12.1)	30 (90.9)	3 (9.1)	29 (87.9)	4 (12.1)
2, *n* (%)	27 (81.8)	6 (18.2)	29 (87.9)	4 (12.1)	33 (100)	0 (0)	10 (57.6)	14 (42.4)	23 (87.9)	10 (12.1)
P	1.00 (NS)		0.73 (NS)		0.11 (NS)		0.004*		0.13 (NS)	

$P<0.05$ is significant; NS: Nonsignificant

of other species of microbes was not statistically significant [Table 3a and b].

The microorganisms isolated from the samples were tested for the antibiotic resistance against few antimicrobials. It was found that tigecycline was the most sensitive against *E. coli*, *Klebsiella*, *Pseudomonas*, and nonfermenting Gram-negative bacteria. The percentage resistance was 14.28% for *E. coli*, 18.18% for *Klebsiella*, 22.44% for *Pseudomonas*, and 22.42% for nonfermenting Gram-negative bacteria. Vancomycin, clindamycin, and tetracycline showed 31.03% resistance against *Staphylococcus* species [Table 4a and b].

Discussion

The threat of cross contamination through pathogenic organisms present in the dental laboratory attire and dental equipment is a growing concern. Although the pathogens isolated from our study included normal oral commensals, hospital environment may act as the source of some other pathogens.

It is indeed difficult to isolate the pathogens and source its origin exclusively. The oral flora has ≥ 300 varieties of microbes including bacteria, fungi, protozoa, and some viruses.[1]

S. aureus is a commensal organism but is also isolated from the following: for skin infections, septicemia, endocarditis, osteomyelitis, pneumonia, and toxic shock syndrome. *E. coli* are normal inhabitants of the human gastrointestinal tract and also isolated from the urinary tract infections and septicemia in some severe cases. *Pseudomonas* and *Klebsiella* are opportunistic pathogens and can cause pneumonia in hospitalized patients. *S. aureus* and coagulase-negative *Staphylococcus* found in the laboratories probably originated from the skin of personnel handling, the prosthesis as they are normal commensals of skin. Hence, isolation of these microbes in the laboratory attire and the equipment may be from the patients coming for the treatments and also from the dental personnel who have not followed the cross infection measures. In varying degrees, cross contamination is a problem which cycles around the staff as well as the patient. The isolation of *Pseudomonas . aeruginosa* and *Klebsiella* in the sample can also bring forward another source of contamination, that is, the water units existing in the hospital setup.

Table 2b: Comparison of presence of nonfermenting Gram-negative bacteria and *Bacillus* spp. in Group 1 and Group 2

Group	NF Gram-negative bacteria		*Bacillus* species	
	Absent	Present	Absent	Present
1, *n* (%)	29 (87.9)	4 (12.1)	28 (84.8)	5 (15.2)
2, *n* (%)	29 (87.9)	4 (12.1)	23 (69.7)	10 (30.3)
P	1.00 (NS)		0.24 (NS)	

*$P<0.05$ statistically significant; $P>0.05$ (NS). NS: Nonsignificant; NF: Nonfermenting

Table 3a: Comparison of presence of (*Staphylococcus aureus*, *Escherichia coli*, and coagulase-negative *Staphylococcus*) in (Group 3-Group 8)

Group	*Staphylococcus aureus*		*Escherichia coli*		Coagulase-negative *Staphylococcus*	
	Absent	Present	Absent	Present	Absent	Present
3, *n* (%)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0)
4, *n* (%)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0)
5, *n* (%)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0)
6, *n* (%)	29 (87.9)	4 (12.1)	31 (93.9)	2 (6.1)	33 (100.0)	0 (0)
7, *n* (%)	27 (81.8)	6 (18.2)	31 (93.9)	2 (6.1)	33 (100.0)	0 (0)
8, *n* (%)	27 (81.8)	6 (18.2)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0)
Fisher's exact value	17.84		5.54		-	
P	<0.001*		0.15 (NS)		-	

*$P<0.05$ is statistically significant; NS: Nonsignificant

Table 3b: Comparison of the presence of *Pseudomonas*, *Klebsiella pneumoniae*, nonfermenting Gram-negative bacteria, and *Bacillus* spp. in (Group 3-Group 8)

Group	*Pseudomonas*		*Klebsiella pneumoniae*		NF Gram-negative bacteria		*Bacillus* species	
	Absent	Present	Absent	Present	Absent	Present	Absent	Present
3, *n* (%)	31 (93.9)	2 (6.1)	31 (93.9)	2 (6.1)	33 (100.0)	0 (0.0)	29 (87.9)	4 (12.1)
4, *n* (%)	27 (81.8)	6 (18.2)	29 (87.9)	4 (12.1)	33 (100.0)	0 (0.0)	31 (93.9)	2 (6.1)
5, *n* (%)	29 (87.9)	4 (12.1)	33 (100.0)	0 (0.0)	29 (87.9)	4 (12.1)	33 (100.0)	0 (0)
6, *n* (%)	27 (81.8)	6 (18.2)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	29 (87.9)	4 (12.1)
7, *n* (%)	25 (75.8)	8 (24.2)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0.0)	33 (100.0)	0 (0)
8, *n* (%)	27 (81.8)	6 (18.2)	31 (93.9)	2 (6.1)	31 (93.9)	2 (6.1)	33 (100.0)	0 (0)
Fisher's exact value	4.972		8.172		9.127		10.368	
P	0.41 (NS)		0.05 (NS)		0.01*		0.02*	

*$P<0.05$ statistically significant, $P>0.05$ (NS). NS: Nonsignificant; NF: Nonfermenting

Table 4a: Percentage of resistance among the isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas*, nonfermenting Gram-negative bacteria) to the antimicrobials

Antimicrobial	*Escherichia coli* (*n*=14), *n* (%)	*Klebsiella* spp. (*n*=22), *n* (%)	*Pseudomonas* spp. (*n*=49), *n* (%)	Nonfermenting Gram-negative bacteria (*n*=14), *n* (%)
Amikacin	9 (64.28)	15 (68.18)	35 (71.42)	9 (64.28)
Azetreonam	7 (50)	15 (68.18)	38 (77.55)	9 (64.28)
Cefotaxime	7 (50)	11 (50)	36 (73.46)	7 (50)
Cefoxitin	5 (35.71)	11 (50)	35 (71.42)	8 (57.14)
Cefuroxime	10 (71.42)	11 (50)	32 (65.30)	7 (50)
Chloramphenicol	8 (57.14)	18 (81.81)	27 (55.10)	12 (85.71)
Ciprofloxacin	8 (57.14)	22 (100)	23 (46.93)	14 (100)
Cotrimoxazole	9 (64.28)	15 (68.18)	28 (57.14)	9 (64.28)
Ertapenem	5 (35.71)	4 (18.18)	28 (57.14)	2 (14.28)
Gentamicin	6 (42.85)	15 (68.18)	24 (48.97)	9 (64.28)
Imipenem	5 (35.71)	4 (18.18)	29 (59.18)	3 (21.42)
Piperacillin tazobactum	5 (35.71)	4 (18.18)	22 (44.89)	3 (21.42)
Tigecycline	2 (14.28)	4 (18.18)	11 (22.44)	3 (21.42)

Table 4b: Percentage of resistance among the isolates *Staphylococcus* spp. to the antimicrobials

Antimicrobial	Staphylococcus spp. (*n*=29), *n* (%)
Amikacin	19 (65.52)
Ampicillin	20 (68.97)
Cefotaxime	17 (58.62)
Cefoxitin	9 (31.03)
Oxacillin	20 (68.97)
Linezolid	17 (58.62)
Erythromycin	17 (58.62)
Cotrimoxazole	19 (65.52)
Vancomycin	9 (31.03)
Gentamicin	12 (41.38)
Clindamycin	9 (31.03)
Tetracycline	9 (31.03)

Various studies have isolated these microbes from the Dental Waterline Units.[2]

This study evaluated the type and the level of microorganism from the dental laboratory attire of the students and dental technicians and the dental equipment. According to the study conducted by Wong *et al*. white coats, nurses uniform, and other hospital garments may play an important role in transmitting pathogenic bacteria in the hospital setting. Dental laboratory attire and dental equipment can also be a source of cross contamination.[3-6] The knowledge of microorganisms present is important in implementing the required sterilization methods and improving the safety of patients.

The sterilization protocol used in the institute is by autoclaving the washed instruments in a cycle of 121°C for 30–90 min. The white coats will be replaced twice every week.

A study conducted by Malini *et al*. on the microbiological analysis of white coat in the dental operatory showed coagulase-negative *Staphylococcus*, *E. coli*, *Pseudomonas* and *Klebsiella*, *Streptococcus viridians*, *micrococci*, and *Enterococcus faecalis*.[4] The present study also had few microorganisms in common except *S. viridians*, *micrococci*, and *E. faecalis*. Nonfermenting Gram-negative bacteria and *Bacillus* species were also found in this study. The level of contamination was more in the dental laboratory attire of the students and dental technicians rather than the dental equipment. This shows that the dental laboratory attire instead of providing a barrier to cross contamination is becoming a source of nosocomial infection. The high rates of the microbial contamination of dental attire may be associated with the following facts: First, the patients continuously shed infectious microorganisms in the hospital environment and the dental students are in constant contact with these patients.[5] Second, it has been demonstrated that microorganisms can survive between 10 and 98 days on fabrics which are used to make the laboratory attire. The microrganisms found in the laboratory attire of dental technicians and dental instruments are more likely to be from the dental casts, impressions, bite records, and environment.[6] Hence, the disinfection of these materials should also be taken into consideration in reducing the risk of nosocomial infections.

As majority of these isolated organisms are not a part of the normal oral microflora, more care should be taken regarding the sterilization protocols administered for the noncritical as well as the critical items. Dental Water Units must be checked for the biofilm formation. According to the American Dental Association and Centers for Disease Control and Prevention guidelines, commercially available options for improving the water quality include the use of independent reservoirs, source water treatment systems, chemical treatment regimens, daily draining, air purging, and pointofuse filters.[7]

There is an increased chance of cross contamination to the patients as the dental prosthesis are fabricated using the dental equipment harboring these microorganisms. The

chances of dental students and dental laboratory technicians getting infected are also very high. As the dental laboratory attire of the students and dental laboratory technicians had increased variety and colony count of microorganisms, it can be concluded that the dental laboratory attire is at high risk for cross contamination than the dental equipment.

The increasing antibiotic resistance to the most commonly used antimicrobials is an alarming situation. The isolated samples of *Klebsiella* and nonfermenting Gram-negative bacteria showed 100% resistance to ciprofloxacin. *Pseudomonas* showed increased resistance to amikacin, azetreonam, cefotaxime, cefoxitin, and cefuroxime. *Staphylococcus* species also showed resistance to most of the antimicrobials. The results in this study were comparable to the study by Asma Banu *et al*., in which most of the Gram-positive cocci were found to be resistant to penicillin, erythromycin, and clindamycin. The most problematic health-care associated multiresistant species are methicillin-resistant *S. aureus*, extended-spectrum beta-lactamases-producing *Enterobacteriaceae*, and carbapenemase-producing Gram-negative bacteria.[8] The studies have shown these microorganisms to be colonizing in dental impressions and gypsum casts.[9,10] Hence, the strict sterilization protocols have to be incorporated in disinfecting the casts and impressions also.

To prevent the transfer of pathogens from dental equipment and dental laboratory attire to the patients and also the dental personnels; the sterilization awareness and methods are needed. Contaminated clinical aprons should be avoided from the laboratory areas. This will reduce the transfer of pathogens to some extent. Sterilization of casts and impressions should also be done before the fabrication of prosthesis.[11] Even a regular disinfection or sterilization of dental laboratory equipment are also required for preventing nosocomial infections.

Conclusion

Within the limitations of the study, it can be concluded that the dental laboratory attire is at high risk for cross contamination than the dental equipment, as the dental laboratory attire of the students and dental laboratory technicians had increased the variety and the colony count of microorganisms. Furthermore, most of the isolated organisms showed increased antimicrobial resistance and majority of the isolated organisms are not a part of the normal oral microflora. The source can be the patient derived as well as hospital environment derived. Source of the normal oral commensals can be attributed to cross contamination of the staff and the patient. Whereas, the other potentially pathogenic microbes can flourish in the biofilms of the Dental Water Units as well as infectious hospital patients. To avoid cross infection, the autoclaved instruments should be handled in a sterile environment, with the use of sterile gloves to promote improved protection. Laboratory attire must be exclusively used in the clinical setup. With all these measures, a strict sterilized environment can be maintained.

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Conflicts of interest

There are no conflicts of interest.

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