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ORIGINAL ARTICLE

Bacterial indoor-air load and its implications for healthcare-acquired infections in a teaching hospital in Ethiopia

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Abstract

Lack of regular cleaning and disinfection practices of the hospital environment is among the main factors for the spread of healthcare-acquired infections (HAIs). The aim of this study is to determine bacterial indoor-air load and antimicrobial susceptibility pattern of isolates from rooms of Adama Hospital Medical College in Ethiopia. A cross-sectional study was conducted from May to August 2013. A total of 78 indoor-air samples were collected from 29 rooms of the hospital. Using passive air sampling method, a 90mm diameter Petri-plate containing Sheep Blood agar (Oxoid, UK) was left open according to the 1/1/1 scheme. The samples were processed following standard bacteriological procedures at diagnostic bacteriology unit, Oromia Public Health Laboratory (OPHRCBQAL). Data were analyzed using SPSS version 16.0. Overall, 182 bacterial isolates were recovered with an average of 3.42 bacterial species/room. The predominant isolates were coagulase negative staphylococci (CNS) (42.9%), followed by *Staphylococcus aureus* (20.3%), *Pseudomonas* spp. (10.4%), *Escherichia coli* (6.6%) and *Salmonella* spp. (6%). The highest mean colony forming units was obtained in obstetrics and surgical wards. Eight percent of the *S. aureus* and 7.6% of the CNS were resistant to 8 and 7 classes of antibiotics including meticillin, respectively. The indoor-air bacterial load of the hospital rooms was beyond the acceptable standard. Profile of the isolates revealed the presence of multidrug resistant agents that may cause HAI. Hence, safety precautions should be strictly followed in the hospital to prevent tragic outcomes of HAIs.

Key words: Indoor air quality; Bacteria; Healthcare associated infection

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Background

Healthcare acquired infections (HAIs) are infections acquired in healthcare facilities while the patient stays in the health facility for 48hrs or more. It is a global health concern, with an estimate of more than 1.7 million people suffering from HAIs annually in US alone.[1] About 5% to 10% of patients admitted to modern hospitals in the developed countries acquire one or more HAIs.[1,2] In the developing countries, magnitude of HAIs is much higher.[3,4] In sub-Saharan Africa, prevalence of HAIs ranges from 2.5% to 14.8%, with cumulative incidence in surgical wards being very high.[3-5]

Various etiologic agents cause HAIs, more than 90% of which are due to bacteria.[6,7] Healthcare equipment and the hospital environments are potential sources of the microorganisms causing HAIs. In the absence of regular disinfection practices, contamination of patient-care medical equipment and the healthcare environment with microorganisms is inevitable.[8,9] In spite of this, infection prevention protocols are hardly followed by healthcare workers (HCWs) in developing countries.[10-12] Besides keeping microbial quality of healthcare equipment, regulation of indoor-air of the rooms is essential for the well-being of the occupants. Air contaminated with bacteria remains without intervention when compared to surface microbial contaminants, and this could be a source of infection for the patients and HCWs.

Despite the significant role of indoor-air contamination to HAIs, microbial profile of indoor-air in healthcare facilities in Ethiopia is not well explored. Studies on bacteriological quality of indoor-air of healthcare facilities in Ethiopia are scarce, and the few available ones documented unacceptably high indoor-air bacterial load.[12,13] Potentially pathogenic bacterial isolates were also detected from healthcare equipment.[10] Nationally, infection prevention guideline has been developed for healthcare facilities.[14] However, adherence of the HCWs to the protocol is questionable.[15] This in turn may play a role for poor microbiological indoor-air quality in the hospitals.[16] The aim of this study is to determine indoor-air bacterial load in rooms of Adama Hospital Medical College (AHMC).

Methods

Study setting and design

A cross-sectional study was conducted from May to August 2013 at AHMC in Adama town. Adama town is located 99Kms east of Addis Ababa, Ethiopia. AHMC is a public teaching hospital, established in 1953. It has 300 beds capacity, serving an estimated 10 million people in Oromia and surrounding regions.

Indoor-air sampling

Seventy-eight indoor-air samples were collected from 29 rooms of the hospital. These include the Operating Room (OR), Surgical ward (SW) and Obstetrics ward (ObW) and Neonatal care unit (NCU). Based on daily human trafficking, samples were collected three times in a day; morning (8:00am-9:00am), midday (11:00am to 12:00am) and evening (5:00pm to 6:00pm) in the SW, ObW and NCU. ORs were classified in to three different zones as described elsewhere[17] as critical zone (operation theater room), semi-critical zone (corridors, recovery and clothing rooms) and non-critical zone (sterilization and store rooms). The samples were collected during active and passive time of the OR.

Specimen collection and identification techniques

Passive (settle plate) air sampling method was employed to determine the Index of Bacterial Air contamination (IBA). IBA corresponds to the number of colony forming units (CFUs) count on a Petri-plate with a diameter of 90mm left open according to the 1/1/1 scheme (for 1hr, at 1m above the floor and 1m away from walls or any major obstacles).[18,19] The IBA per Petri-plate for total viable colony count was counted in CFU/dm^2/hr and CFU count was converted to CFUm^3 according to Polish Standard PN 89/z-04008/08. A 5% defibrinated Sheep Blood agar (Oxoid, UK) plate was used to recover all possible indoor-air bacterial colonizers. After the plates were left open according to the 1/1/1 scheme, they were covered back with their lids. The plates were placed in sterile container and transported to Oromia Public Health Research, Capacity Building and Quality Assurance Laboratory (OPHRCBQAL), which is located next to the hospital. The plates were incubated at 37°C for 48hrs. After

48hrs of incubation, colony counting, characterization and identification were performed following standard bacteriological techniques.[20] Anaerobic gram-positive cocci isolates were initially identified based on colony characterization, haemolysis pattern and gram staining of the colonies. Further identification was made with Catalase test, Mannitol fermentation and Coagulase test. Gram-negative bacilli were identified using the following tests; catalase, oxidase, urease, glucose and lactose fermentation, citrate utilization, lysine decarboxylation, indole, gas and H_2S production and motility tests.[20] All biochemical test reagents were obtained from Oxoid, UK.

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed using the modified Kirby-Bauer disk diffusion technique.[21] Bacterial suspension turbidity was adjusted to 0.5McFarland standard. Antimicrobial agents used were; penicillin (10iu), ceftriaxone (30µg), cefoxitin (30µg), erythromycin (10µg), clindamycin (10µg), co-trimoxazole (25µg), vancomycin (30µg), chloramphenicol (30µg), tetracycline (30µg), gentamicin (30µg), ampicillin (10µg), piperacillin (100µg), nitrofurantoin (300µg), nalidixic acid (30µg) and ciprofloxacin (1µg); all were from Oxoid, UK. Cefoxitin (30µg) disc was used for the detection of MRSA. Antimicrobial agents were selected based on clinical significance, local treatment protocol and literature data search. The results were interpreted using Clinical Laboratory Standards Institute (CLSI) guideline. The control strains *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922) and *P. aeruginosa* (ATCC 27853), which were kindly provided from Ethiopian Public Health Institute, were used to check the potency of antimicrobial discs.[20,22]

Statistical analysis

Data were cleaned and entered into the computer, and analyzed using SPSS version 16.0. Descriptive statistics were used to summarize the data. Indoor-air bacterial load in the different rooms of the hospital was compared using Chi-square test. *P*-value of <0.05 was considered as statistically significant.

Ethical clearance

Ethical clearance was obtained from AHMC Ethical Review Board. Permission was sought from the hospital administration before starting sample collection.

Results

Description of the rooms

A total of 78 indoor-air samples were collected from 29 rooms and investigated for bacterial profile and load. Thirty-three (42.3%), 24 (30.8%), 18 (23.1%), 3 (3.8%), of the indoor-air samples were collected from 11 SWs, 8 ObWs, 9 ORs and a NCU, respectively. Of the 33 SW samples, 55% and 45% were collected from 6 male and 5 female SWs, respectively.

Bacterial contamination

The observed bacterial indoor-air load of the sampled rooms is presented in table I. Bacterial indoor-air load in all of the investigated rooms was considerably higher than the acceptable limit. Out of the total rooms included in this study, the highest average count was observed in ObWs and SWs with 12053±1831.1cfu/m^3 and 8792.1±2943.7cfu/m^3, respectively. The average indoor-air load in OR critical zones during surgery (active time) and no surgery (passive time) was 4124±2064.6cfu/m^3 and 2889.1±1288.5cfu/m^3, respectively.

Mean bacterial indoor-air load in the samples collected from the rooms at different period is shown in figure 1. The highest mean bacterial CFUs counts were observed at midday's in the investigated rooms.

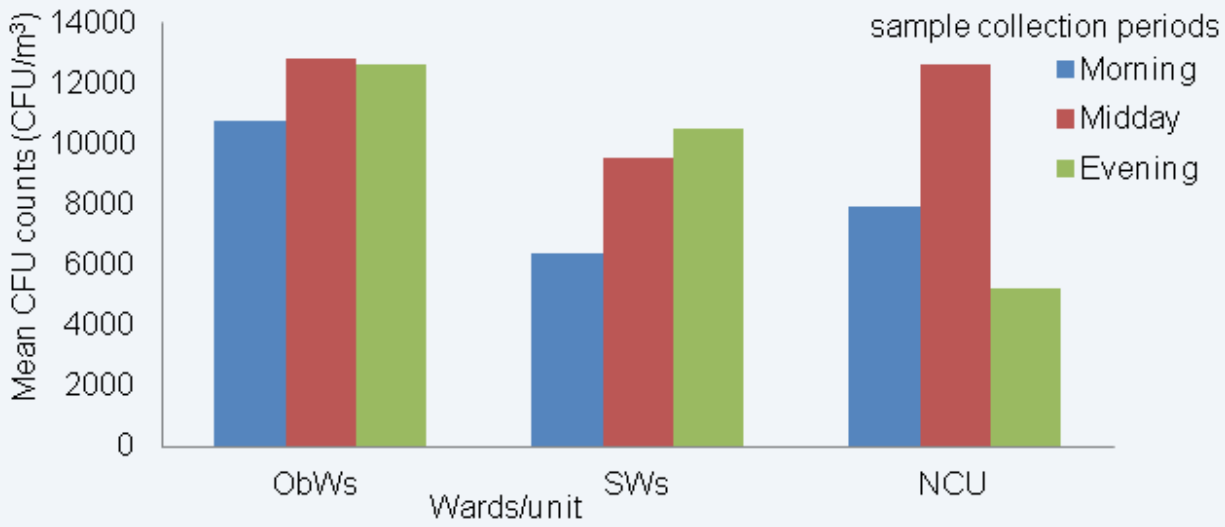
Bacterial isolates

Profile of bacterial isolates detected in the study is shown in figure 2. A total of 182 bacterial isolates were recovered from indoor-air samples of the investigated rooms. The maximum number of bacterial species isolated from a single room was 6 (average of 3.42 bacterial species per room). Out of the total isolates, coagulase negative staphylococci (CNS) was predominant, which accounts for 78 (42.9%) of the isolates followed by *S. aureus* (20.3%). Other isolates

Table I. Indoor-air bacterial load in different rooms of AHMC, 2013

Rooms		Total Microbial Count			
		Standard[18,19] (CFU/dm²/hr)		Observed Mean ±SDV	
		Acceptable	Unacceptable	(CFU/dm²/hr)	(CFU/m³)
SWs		251–450	> 451	671.33 ±163.5	8792.1±2943.7
ObWs		251–450	> 451	920±85.9	12053±1831.1
NCU		51–90	> 91	653±284.6	8558.1±3729.7
OR-Critical Zone	Active	61–90	> 91	314.67±157.5	4124±2064.6
	Passive	5–8	> 9	220.44±98.3	2889.1±1288.5

Key: SW-Surgical Ward; ObW-Obstetrics Ward; OR-Operating Room; CFUs-Colony Forming Units; NCU- Neonatal Care Unit; SDV-Standard Deviation



Key: *-SWs- Surgical Wards; ObWs- Obstatric Wards; NCU- Neonatal Care Unit; CFU- Colony Forming Units*

Figure 1. Mean bacterial CFUs in the wards/unit at different periods of sample collection at AHMC, 2013

include *Pseudomonas* spp. (10.4%), *E. coli* (6.6%), *Salmonella* spp. (6.6%) and *Klebsiella* spp. (4.9%).

Of the 182 bacterial isolates, 104 (57.1%) were potential pathogens. Pathogenic bacterial isolates were higher in ObWs (63.2%) and SWs 49 (59.8%) compared to the other wards. However, the difference was not significant (p>0.05). Nearly two-third (65.2%) of bacteria isolated during midday of the investigations were potential pathogens. The pathogenic pattern of the bacterial isolates is presented in table II.

Antibiotic susceptibility pattern

The antibiotic resistance pattern of the bacterial isolates is shown in table III. The bacterial isolates were tested for 14 different antibiotics. Gram-positive isolates showed the highest resistance, with 88.5% of the CNS and 86.5% of the *S. aureus* being resistant to penicillin. Just over half (51%) of the *S. aureus* and 39.7% of the CNS were also resistant to erythromycin. Thirteen (16.7%) of the CNS and 10 (27%) of the *S. aureus* isolates were resistant to meticillin. However, none of them were resistant to vancomycin. Of the

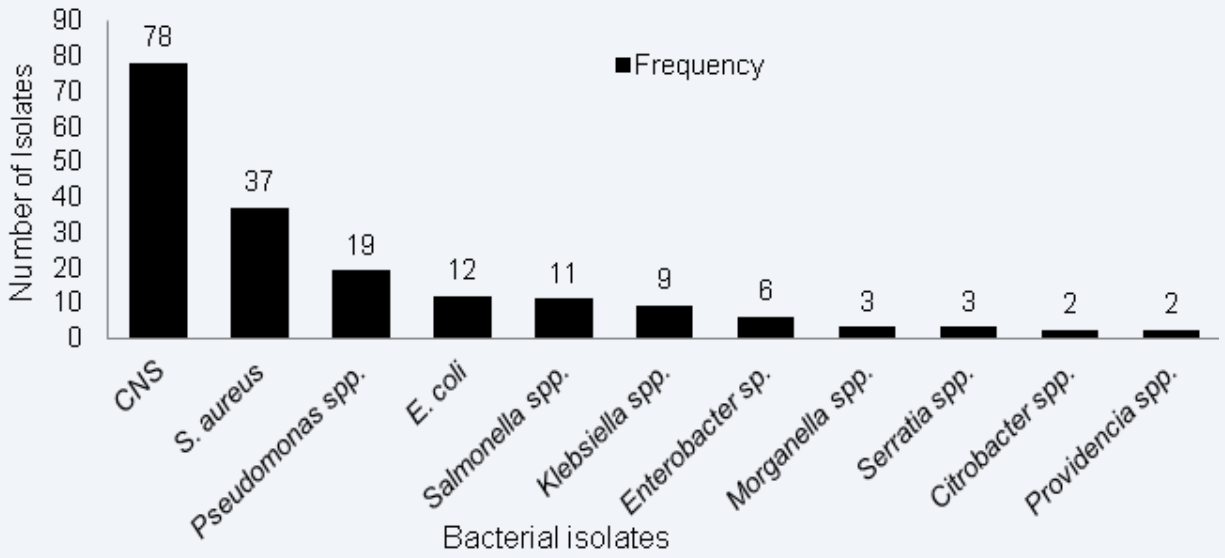


Figure 2. Frequency of bacterial isolates from indoor-air of different rooms at AHMC, 2013

gram-negative isolates, *Citrobacter, Serratia,* and *Providencia* species showed 100% resistance to ampicillin. Moreover, 91.7% and 88.9% of the *E. coli* and *Klebsiella* species were resistant to ampicillin, respectively.

Some of the bacterial isolates obtained in this study were multidrug resistant. Of the gram-positive isolates, 6 (7.6%) of the CNS and 3 (8.1%) of the *S. aureus* isolates were resistant to 7 and 8 classes of antibiotics, respectively. Similarly, 33.3% of the *E. coli* and *Klebsiella* species were resistant to six antibiotic classes each (table IV).

Discussion

This study revealed that bacteriological quality of indoor-air of the investigated wards and ORs of the hospital was far beyond the acceptable limit.[18,19] As compared to earlier studies done by Genet *et al.*[12] and Akinyemi *et al.*,[23] the finding in this study shows higher degree of indoor-air contamination of the hospital rooms. The average CFU in the OR during active (surgery) and passive (rest) times obtained in this study was three times higher than the acceptable limit. The higher bacterial load compared to the standard could be an indicator of lack of routine disinfection practices in the rooms. The high load of indoor bio-aerosols apparently put the occupants at an increased risk of infection. Particularly, at working time of the operation theater, like other scholars[24,25] we agree that such settle plates are potential indicator of wound contamination rate. Since, it shows the real scenario, when there is no routine disinfection practice; contaminating microbes and dust particles aerosolize and bind with each other then sediments on open wound during surgery or during wound dressing. The development of HAIs may extend patient days spent at the hospital, which may also have economic implications. Enclosed space confines and protects the contaminated aerosols in the room.[26] The higher bacterial indoor-air load could be due to the uncontrolled human traffic in the rooms, poor ventilation system and overcrowding of the beds. This finding calls for urgent infection prevention practices to be strictly followed, and regular monitoring of indoor-air quality.

In this study, the highest and lowest bacterial CFUs were obtained in samples collected at midday and morning, respectively. Similar finding was reported earlier in Jimma.[12] High bacterial indoor-air load during midday might be due to higher human traffic (both visitors and personnel) in the rooms during these times, which could initiate aerosolization of dust particles resulting in binding of the particles to the suspended microbes in the air and fallout in numbers due to gravitation.

The average number of bacterial species isolated in the rooms was 3.42, with maximum of 6 bacterial

Table II. Pathogenicity of the bacterial isolates from indoor-air of the wards/units and periods of sample collection, AHMC, 2013

Parameters		Pathogenicity		
		Potential Pathogen n (%)	Opportunistic n (%)	p-value
Rooms	SW	49 (59.8)	33 (40.2)	0.81
	ObW	42 (63.6)	24 (36.4)	
	OR	10 (35.7)	18 (64.3)	
	NCU	3 (50)	3 (50)	
Periods of sample collection	Morning	35 (59.3)	24 (40.7)	0.78
	Midday	30 (65.2)	16 (34.8)	
	Evening	29 (59.2)	20 (40.8)	
	Passive	2 (18.2)	9 (81.8)	0.11*
	Active	8 (47.1)	9 (52.9)	
	Total	**104 (57.1)**	**78 (42.9)**	

Key: *- Fisher exact test; n-number, SW-Surgical Ward; ObW-Obstetrics Ward; OR-Operation Room; NCU- Neonatal Care Unit

species per room. Similar to most of the previous studies,[9,23,26,27] CNS was the most frequently detected isolate in this study followed by *S. aureus*. Similar species of bacteria were reported from studies done in Pakistan[28] and Mexico.[29] Among the bacteria isolated in this study, 44% were potential pathogens. Surgical wards and ObWs contained higher load of potentially pathogenic bacteria compared to the other rooms, however the difference was not statistically significant (P>0.05). Higher mean CFUs of potentially pathogenic bacteria were detected in indoor-air samples collected at midday; however, there was no significant difference in mean bacterial CFUs with periods of sample collection.

Large proportions of CNS (88.5%) and *S. aureus* (86.5%) isolated in this study were resistant to penicillin, which is locally the antibiotic of choice for the treatment of infections caused by these bacteria. A similarly high prevalence of resistance of CNS and *S. aureus* to penicillin-G has also been reported from Jimma in Ethiopia.[12] This study also revealed that 16.7% of the CNS and 27% of the *S. aureus* isolates were resistant to meticillin. However, all the meticillin resistant *S. aureus* (MRSA) isolates were sensitive to vancomycin. MRSA strains are known to survive on dry surfaces for long period of time,[30] which might have contributed to the spread of this organism. All the *Citrobacter* spp., *Serratia* spp. and *Providencia* spp. displayed resistance to ampicillin. Majority of the *E. coli* and *Klebsiella* species were also resistant to ampicillin. Similar pattern of resistance to ampicillin has been reported earlier.[10] Ampicillin is one of the most commonly prescribed antibiotic forms in healthcare facilities in Ethiopia.[31] Irrational prescription of the antibiotic and its misuse by patients might have contributed for the resistance of most of the isolates to the antibiotic.

Conclusions

The degree of bacterial indoor-air loads were far beyond the acceptable limits in the rooms. Microbiological profiles of the isolates were worrisome due to: (i) the presence of diversified pathogenic and well known causative agents of HAI in highly sensitive hospital environments, including operating theater rooms and neonatal care units and (ii) the resistance pattern of the isolates to commonly prescribed antibiotics.

Therefore, awareness creation among the healthcare professionals on the pattern and consequences of HAIs and antibiotic resistance is recommended. Regular surveillance of bacteriological quality of indoor-air of the hospital rooms is required. Moreover, HCWs should adhere to the regular cleaning and disinfection

Table III. Antibiotic resistance pattern of the isolates from indoor-air of different rooms at AHMC, 2013

Antibiotics (µg)	Bacterial Isolates										
	CNS n (%)	S. aureus n(%)	E. coli n(%)	Klebsiella spp. n(%)	Pseudomonas spp. n(%)	Salmonella sp. n(%)	Enterobacter spp. n(%)	Citrobacter spp. n(%)	Serratia spp. n(%)	Morganella spp. n(%)	Providencia spp. n(%)
FOX (30)	13 (16.7)	10 (27)	ND	ND	ND	ND	ND	ND	ND	ND	ND
V (30)	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND
P (10IU)	69 (88.5)	32 (86.5)	ND	ND	ND	ND	ND	ND	ND	ND	ND
DA (2)	28 (35.9)	7 (18.9)	ND	ND	ND	ND	ND	ND	ND	ND	ND
E (15)	31 (39.7)	19 (51.4)	ND	ND	ND	ND	ND	ND	ND	ND	ND
CIP (5)	3 (3.8)	2 (5.4)	9 (75)	3 (33.3)	15 (78.9)	5 (50)	2 (33.3)	0	2 (66.7)	0	1 (50)
TE (30)	20 (25.6)	14 (37.8)	7 (58.3)	5 (55.6)	ND	3 (30)	3 (50)	1 (50)	3 (100)	2 (66.7)	1 (50)
C (30)	35 (44.9)	17 (45.9)	7 (58.3)	6 (66.7)	ND	6 (60)	3 (50)	1 (50)	2 (66.7)	1 (33.3)	1 (50)
CN (10)	12 (15.4)	12 (32.4)	0	2 (22.2)	14 (73.7)	4 (40)	1 (16.7)	1 (50)	1 (33.3)	2 (66.7)	1 (50)
TS (25)	9 (11.5)	2 (5.4)	5 (41.7)	2 (22.2)	6 (31.6)	1 (10)	0	2 (100)	2 (66.7)	0	0
CRO (30)	ND	ND	9 (75)	5 (55.6)	ND	4 (40)	3 (50)	0	2 (66.7)	2 (66.7)	1 (50)
NA (30)	ND	ND	6 (50)	2 (22.2)	13 (68.4)	5 (50)	1 (16.7)	1 (33.3)	0	0	1 (50)
AP (10)	ND	ND	11 (91.7)	8 (88.9)	ND	3 (30)	2 (33.3)	2 (100)	3 (100)	2 (66.7)	2 (100)
NI (300)	ND	ND	5 (41.7)	4 (44.4)	14 (73.7)	7 (70)	2 (33.3)	0	1 (33.3)	0	0
PIP (100)	ND	ND	ND	ND	6 (31.6)	ND	ND	ND	ND	ND	ND
Total Isolates	**78**	**37**	12	9	19	11	6	2	3	3	2

Key: ND- not done; N-number; %-percentage; C-chloramphenicol; CIP-ciprofloxacin; CN-gentamicin; DA-clindamycin; P-penicillin; E-erythromycin; FOX-cefoxitin; TE-tetracycline; AP-ampicillin; NI-nitrofurantoin; PIP-piperacillin; CRO-ceftriaxone; TS-thrimetoprin and sulphamethoxazole; V-vancomycin; NA-nalidixic acid

Table IV. Multiple drug resistance patterns of the bacterial isolates from indoor-air of different rooms at AHMC, 2013

Isolates	Type of Antibiotics	No. of Resistant Isolates/ Antibiotic Classes
CNS	C,E,CN,DA,FOX,P,TE	6/7
S. aureus	C,CIP,CN,DA,E,FOX,P,TE	3/8
***Pseudomonas* spp.**	C,CN,CIP,CTR,NA,TE,TS	8/8
E. coli	AP,C,CIP, CTR,CN,TE	4/6
***Klebsiella* spp.**	AP,C,CIP,NI,TE,NA	3/6
***Serratia* spp.**	AP,C,CIP,CTR,NA,TE,TS	1/7
***Providencia* spp.**	AP,C,CIP,CTR,NA,NI,TE	1/7

Key: CNS-Coagulase Negative staphylococci; C- chloramphenicol; CIP- ciprofloxacin; CN- gentamicin; DA- clindamycin; P-penicillin; E- erythromycin; FOX- cefoxitin; TE- tetracycline; AP- ampicillin; NI- nitrofurantoin; CTR- ceftriaxone; TS- co-trimoxazole; NA- nalidixic acid

practice of the working environment and the patient care equipment with effective detergents and disinfectants. Staff should take appropriate precautions to prevent shedding of microbes, and restrict excessive movement in operation room.

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