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Research Article

CHARACTERIZATION OF BACTERIA ISOLATED FROM HOSPITAL ENVIRONMENT

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A B S T R A C T

Background: One of the foremost relevant public health problems is Healthcare-associated Infections (HAIs), both in high-income and developing countries.Hospital Acquired Infections or Nosocomial infections or healthcare-associated infections (HAIs) are often defined as the infections acquired within the hospital by a patient.

Objective: The objective of this study is to isolation and identification of bacterial pathogens from samples in a hospital and to evaluate their antibiotic resistance and sensitivity pattern

Method: The different samples collected were air sample, swab sample, hand swab andprocessed for culture sensitivity. The culture were subjected to different tests like Gram Stain, Catalase Test, Coagulase Test (Slide and Tube Coagulase Test), Oxidase Test, Indole Test, Citrate Test, TSI (Triple Sugar Iron) Test, Urease Test and Antimicrobial Susceptibility Test

Result: A total of 118 air samples & 179 surface swab samples were collected from different wards and OTs of the hospital, & 15 hand swab samples were collected from various personnel professionals of the hospital.Out of 118 air samples, 42.3% (50/118) were culture positive whereas 57.7 % (68/118) were culture negative. Out of 179 surface swab samples, 38.5% (69/179) were culture positive and 61.5% (110/179) were culture negative. 0ut of the 15 hand swab sample 73.3% (11/15) were culture positive and 26.7% (4/15) were culture negative.

Conclusion: The isolation of pathogenic bacteria from the hospital environment indicates that they can be vehicles for disease transmission. So, there is need for thorough disinfection and conscientious contact control procedures to minimize the spreads of these pathogens.

INTRODUCTION

One of the foremost relevant public health problems is Healthcare-associated Infections (HAIs), both in high-income and developing countries.[6]Hospital Acquired Infections or Nosocomial infections or healthcare-associated infections (HAIs) are often defined as the infections acquired within the hospital by a patient,[7]

- who was admitted for a reason aside from that infection.
- in whom the infection wasn't present or incubating at the time of admission.
- symptoms should appear a minimum of 48 hours after admission.
- This includes infections acquired within the hospital but appearing after discharge, and also occupational infections among the staff of the hospital facility.

CDC (Centre for Disease Control and Prevention, Atlanta) had established the National Nosocomial Infections Surveillance (NNIS) program to watch the incidence of nosocomial infections.

It is estimated that 5-10 % of patients admitted to acute care hospitals develop HAIs. Treatment of those HAIs adds a huge economic burden to the hospital.[8]

The principal factors that determine the likelihood that a given patient would acquire a nosocomial infection are:

- Immune status
- Hospital environment
- Hospital Organisms
- Diagnostic or therapeutic interventions
- Transfusion
- Poor Hospital Administration

The aim of this study isthe isolation and identification of bacterial pathogens infection from surveillance samples in a hospital and to evaluate their antibiotic resistance and sensitivity pattern.

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MATERIAL AND METHODS

The study was conducted in Rama Medical College-Hospital & Research Centreon the specimen isolated from the hospital environment.

Sample processing

Air sample

Open blood agar plateswere exposed to air for at least one hour; then the plates were incubated at 37^0C for twenty-four hrs and also the number of colonies was counted.[9]

Surface Swab Sample

Surface swabs were collected from different location in OT and ICU and Wards using peptone water and Robertson Cooked Meat media (RCM).[10]

Hand Swab

Using sterile cotton swabs, hands of OT and ICU, personnel were swabbed and inoculated on blood agar and Mac Conkey agar and incubated for twenty-four hrs, then the isolates were identified and sensitivity was performed.[11]

The following test were performed:

- Gram Stain
- Catalase Test
- Coagulase Test (Slide and Tube Coagulase Test)
- Oxidase Test
- Indole Test
- Citrate Test
- TSI Test
- Urease Test
- Antimicrobial Susceptibility Test

ANTIBIOTICS: (Hi-Media)

- Penicillin 10 µg
- Co-Trimoxazole 25 µg
- Ampicillin 10 µg
- Cefotaxime 30 µg
- Clindamycin 2 µg
- Gentamicin 10 µg
- Tetracycline 30 µg
- Erythromycin 15 µg

RESULTS

In this study, a total of 118 air samples & 179 swab samples were collected from different wards and OTs of the hospital, & 15 hand swab samples were collected from various OT, ICU and ward personnel of the hospital.

Air Samples

Out of 118 air samples, 42.3% (50/118) were culture positive whereas 57.7 % (68/118) were culture negative.

Maximum isolation percentage was from air samples of ICCU, Emergency ward, Ortho dressing room, Psychiatry ward, Gyne. ward, TB ward, Orthopedic ward, Male & Female Surgery ward & ENT ward (100 % each).

The minimum isolation percentage was from air samples of the Ophthalmology ward (00 %).

Out of total positives, 28% sample (14/50) were gram-positive bacteria and 72% (36/50) were gram-negative bacteria.

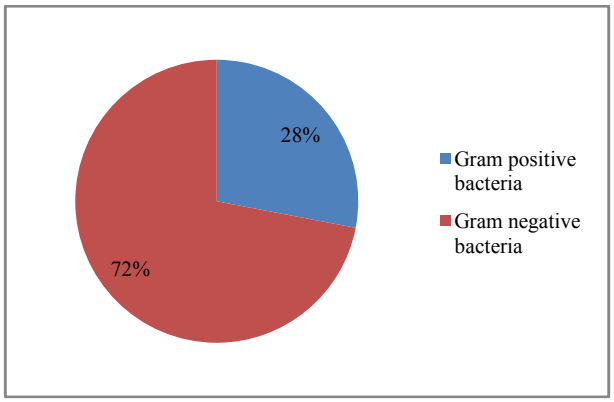

Fig No 1 Total positive isolate of air samples

The overall most common isolate was Escherichia coli in air samples.

One sample from the Emergency ward showed two organisms Klebsiella pneumoniae and Staphylococcus spp.

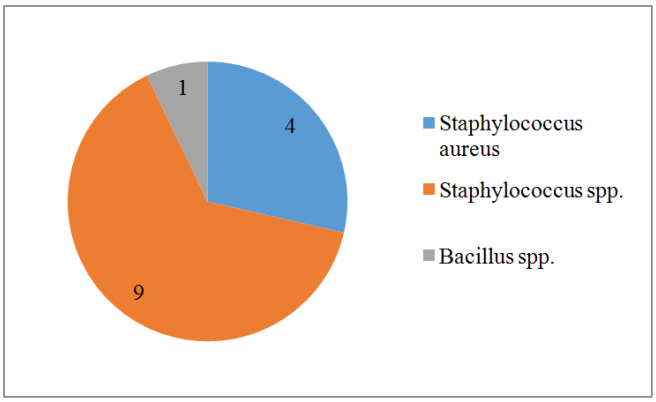

Fig No 2 Gram-positive bacteria in air samples

The most common gram-positive bacteria isolated was Coagulase Negative Staphylococcus species (64.2% (9/14) followed by Staphylococcus aureus (28.5 % (4/14).

Table No 1 AST of Gram-positive bacteria from air sample

Bacteria	Antibiotics	Penicillin	Co-Trimoxazole	Ampicillin	Clindamycin	Vancomycin	Tetracycline	Erythromycin	Gentamicin
Staphylococcus aureus (4)	S	3	2	2	1	4	1	3	1
	I	0	0	0	1	0	1	1	1
	R	1	2	2	2	0	2	0	2
CONS (9)	S	6	6	3	5	9	2	3	5
	I	3	1	1	2	0	3	1	2
	R	0	2	5	2	0	4	5	2

* S-sensitive I-intermediate R-resistant

75% isolates of Staphylococcus aureus were sensitive to Penicillin, whereas 50% of the isolate were sensitive to Co-trimoxazole, Ampicillin, Clindamycin, Tetracycline & Gentamicin. All isolates were found to be sensitive to Vancomycin & Erythromycin.

77.7% isolates of Coagulase Negative Staphylococcus spp. were sensitive to Gentamicin, Clindamycin & Co-trimoxazole, and 55.5% of the isolate were sensitive to Tetracycline, whereas 44.4% of isolates were sensitive to erythromycin &

Ampicillin. All isolates were found to be sensitive to Vancomycin & Penicillin.

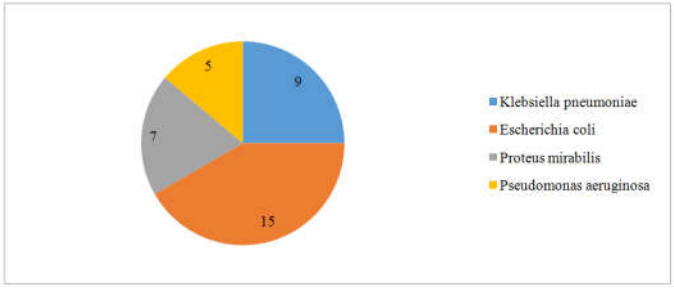

Fig No 3 Gram-negative bacteria in air samples

The most common gram-negative bacteria isolated was Escherichia coli 41.6% (15/36) followed by Klebsiella pneumoniae 25% (9/36), Proteus mirabilis 19.4 (7/36), and Pseudomonas aeruginosa 13.8% (5/36)

Table No 2 AST of Gram-negative bacteria from air samples

Bacteria	Antibiotics	Co-Trimoxazole	Ampicillin	Cefotaxime	Tetracycline	Gentamicin
Escherichia coli(15)	S	2	6	4	11	9
	I	0	2	4	4	6
	R	13	7	7	0	0
Klebsiella pneumoniae (9)	S	5	3	2	3	7
	I	4	1	2	3	2
	R	0	5	5	3	0
Proteus mirabilis (7)	S	3	3	2	0	2
	I	1	1	2	0	0
	R	3	3	3	7	5
Pseudomonas aeruginosa (5)	S	1	2	3	0	1
	I	2	1	0	0	0
	R	2	2	2	5	4

* S-sensitive I-intermediate R-resistant

53.3% isolates of Escherichia coli were sensitive to Ampicillin & Cefotaxime whereas 13.3% of the isolate were sensitive to Co-trimoxazole. All isolates were found to be sensitive to Tetracycline & Gentamicin.

66.6% isolates of Klebsiella pneumoniae were sensitive to tetracycline, whereas 44.4% of the isolate were sensitive to Cefotaxime & Ampicillin. All isolates were found to be sensitive to Co-trimoxazole & Gentamicin.

57.1% isolates of Proteus mirabilis were sensitive to Co-trimoxazole, Cefotaxime & Ampicillin, whereas 28.5% of the isolate were sensitive to Gentamicin.

60% of isolates of Pseudomonas aeruginosa were sensitive to Co-trimoxazole, Cefotaxime & Ampicillin whereas 20% of the isolate were sensitive to Gentamicin.

Surface Swab Samples

Out of 179 swab samples, 38.5% (69/179) were culture positive and 61.5% (110/179) were culture negative.

The maximum isolation percentage was from swab samples of the Ophthalmology ward, TB ward & Psychiatry ward (66.6% each).
The minimum isolation percentage was from swab samples of MICU & high-risk labor Room (20% each.)Out of total positives, 30.5% samples (21/69) were Gram-Positive bacteria and 69.5% (48/69) were Gram-negative bacteria.

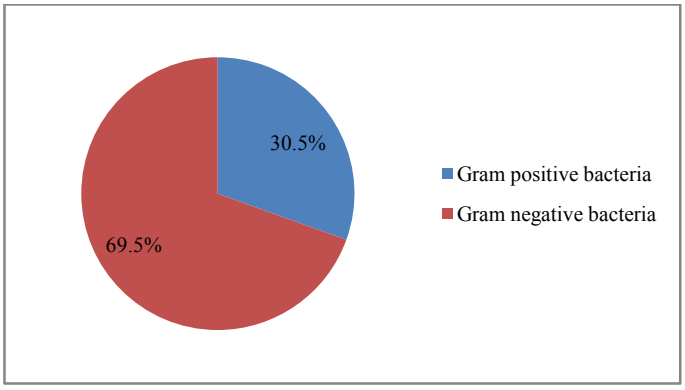

Fig No 4 Total positive isolates of swab samples

The overall most common isolate was Klebsiella pneumoniae. One sample from the General surgery Dressing Room showed two organisms Klebsiella pneumoniae and Staphylococcus spp.

One sample from the TB ward showed two organisms Proteus mirabilis and Staphylococcus spp.

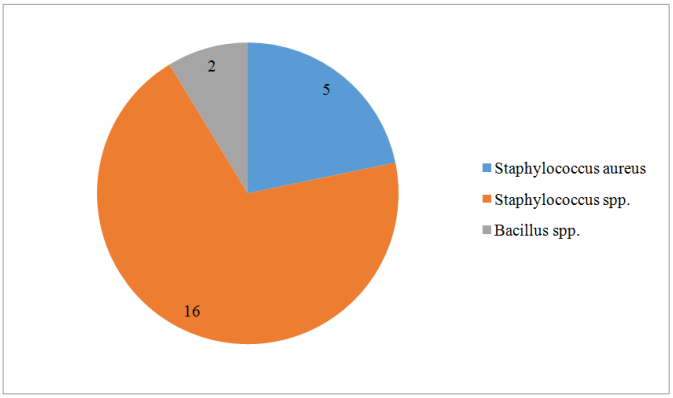

Fig No 5 Gram-positive bacteria in surface swab samples

The most common Gram-positive bacteria isolated was Coagulase Negative Staphylococcus species 7.1% (16/21) followed by Staphylococcus aureus 23.8% (5/21).

Table No 3 AST of Gram-positive bacteria from surface swab samples

Bacteria	Antibiotics	Penicillin	Co-Trimoxazole	Ampicillin	Clindamycin	Vancomycin	Tetracycline	Erythromycin	Gentamicin
Staphylococcus aureus (5)	S	2	2	2	0	5	0	4	2
	I	0	1	0	0	0	0	1	0
	R	3	2	3	5	0	5	0	3
CONS (16)	S	5	7	6	5	16	7	8	4
	I	2	2	2	1	0	3	0	1
	R	9	7	8	10	0	6	8	11

* S-sensitive I-intermediate R-resistant

60% of isolates of Staphylococcus aureus were sensitive to Co-trimoxazole, whereas 40% of the isolate were sensitive to Penicillin, Ampicillin & Gentamicin. All isolates were found to be sensitive to Vancomycin & erythromycin
62.5% isolate of Coagulase Negative Staphylococcus spp. were sensitive to tetracycline, 56.2% of the isolate were sensitive to Co-trimoxazole, 50% of the isolate were sensitive

to Ampicillin & Erythromycin, 43.7 % of the isolate were sensitive to Penicillin, 37.5% of the isolate were sensitive to Clindamycin, whereas 31.2% of the isolate were sensitive to Gentamicin. All isolates were found to be sensitive to Vancomycin.

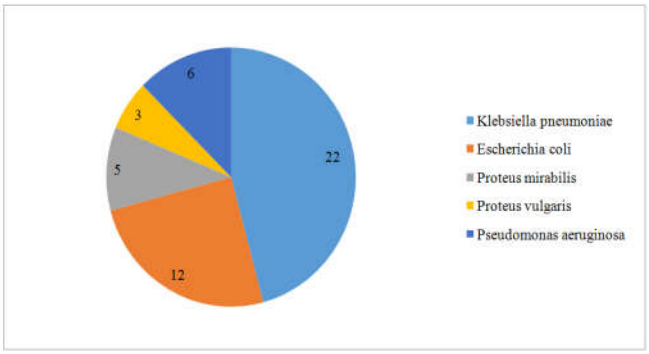

Fig No 6 Gram-negative bacteria in surface swab samples

The most common Gram-negative bacteria isolate was Klebsiella pneumoniae 45.8% (22/48) followed by Escherichia coli 25% (12/48), Pseudomonas aeruginosa 12.5% (6/48), Proteus mirabilis 10.4% (5/48) & Proteus vulgaris 6.2% (3/48).

Table No 4 AST of Gram-negative bacteria from surface swab samples

Bacteria	Antibiotics	Co-Trimoxazole	Ampicillin	Cefotaxime	Tetracycline	Gentamicin
Klebsiella pneumoniae (22)	S	10	7	6	3	15
	I	4	2	3	1	7
	R	8	13	13	18	0
Escherichia-coli (12)	S	6	5	4	7	9
	I	2	1	2	0	3
	R	4	6	6	5	0
Proteus mirabilis (5)	S	3	1	2	0	2
	I	1	1	0	0	1
	R	1	3	3	5	2
Proteus vulgaris (3)	S	1	2	1	0	2
	I	0	0	1	0	0
	R	2	1	1	3	1
Pseudomonas aeruginosa (6)	S	3	3	4	2	0
	I	0	1	0	0	0
	R	3	2	2	4	6

* S-sensitive I-intermediate R-resistant

63.6% isolates of Klebsiella pneumoniae were sensitive to Co-trimoxazole, 40.9% of the isolate were sensitive to Ampicillin & Cefotaxime whereas 18.1% of the isolate were sensitive to tetracycline. All isolates were found to be sensitive to Gentamicin.

66.6% isolates of Escherichia coli were sensitive to Co-trimoxazole, 58.3% of the isolate were sensitive to Tetracycline, whereas 50% of the isolate were sensitive to Ampicillin & Cefotaxime. All isolates were found to be sensitive to Gentamicin. 80% isolates of Proteus mirabilis were sensitive to Co-trimoxazole, 60% of the isolate was sensitive to Gentamicin, whereas 40% isolate was sensitive to Ampicillin & Cefotaxime. 66.6% isolates of Proteus vulgaris were sensitive to Ampicillin, Gentamicin & Cefotaxime, whereas 33.3% of the isolate were sensitive to Co-trimoxazole. 66.6% isolates of Pseudomonas aeruginosa were sensitive to Ampicillin & Cefotaxime, 50% of the isolate were sensitive to Co-Trimoxazole, whereas 33.3% of the isolate were sensitive to Tetracycline. Maximum isolation percentage was from swab sample of warmer (100%). Minimum isolation percentage was from swab sample of Instrument Trolley, Laparoscopy Machine & X-Ray View Box (00%) each.

Hand Swab Sample

0ut of the 15 hand swab sample 73.3% (11/15) were culture positive by 26.7% (4/15) were culture negative. Out of the total positive 90.9% sample (10/11) were gram-positive bacteria and 9.1% (1/11) were gram-negative bacteria.

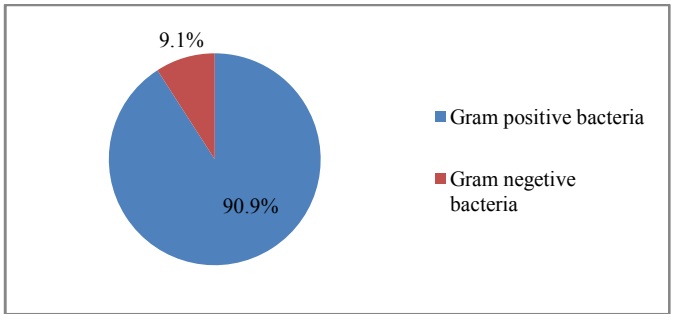

Fig No 7 Total positive isolate of hand swab samples

Overall most common isolate is Coagulase Negative Staphylococcus spp. One sample showed two organisms Proteus vulgaris & Coagulase Negative Staphylococcus spp..

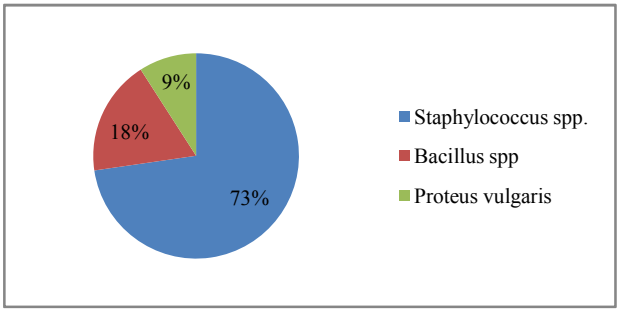

Fig No. 8 Gram-positive & Gram-negative bacteria in hand swab samples

The most common gram-positive bacteria isolate was Coagulase Negative Staphylococcus spp. 80% (8/10).

Table No 5 Distribution of Organism

Organism	No.	Percentage
Bacillus species	02	18.1 %
Coagulase Negative Staphylococcus spp.	08	72.7 %
Proteus vulgaris	01	9.1 %
Total	11	100 %

Table No 6 AST of Gram-positive & negative bacteria from hand swab samples

		Penicillin	Co-Trimoxazole	Ampicillin	Clindamycin	Vancomycin	Tetracycline	Erythromycin	Gentamicin	Cefotaxime
Cons (8)	S	4	3	4	3	8	3	3	4	-
	I	0	1	1	0	0	1	1	1	-
	R	4	4	3	5	0	5	4	3	-
Proteus vulgaris(1)	S	-	1	0	-	-	1	-	1	0
	I	-	0	0	-	-	0	-	0	0
	R	-	0	1	-	-	0	-	0	1

* S-sensitive I-intermediate R-resistant

62.5% isolates of Coagulase Negative Staphylococcus spp. were sensitive to Gentamicin, 50 % of the isolate were sensitive to Penicillin, Co-trimoxazole, Tetracycline & Erythromycin whereas 37.5% of the isolate were sensitive to Clindamycin. All isolates were found to be sensitive to Vancomycin.

Proteus vulgaris was sensitive to Co-trimoxazole, Tetracycline & Gentamicin and resistant to Ampicillin & Cefotaxime.

Table No 7 Different samples

S. No.	Sample	Total No.	Positive	Negative	Positive%
1.	Air sample	118	50	68	42.3 %
2.	Surface Swab sample	179	69	110	38.5 %
3.	Hand Swab sample	15	11	4	73.3 %

DISCUSSION

Air Sample

The most common isolate was Escherichia coli as was in the study conducted by Bhandari P, Thapa G, *et al*, 2015[12]. But in the study conducted by Mogs F, Endris M, *et al*, 2014[13]the most common isolate was Klebsiella Pneumoniae.

Escherichia coli was highly resistant to Co-Trimoxazole & highly sensitive to Tetracycline & Gentamicin, whereas in the study conducted byMogs F, Endris M, *et al*2014[13], Escherichia Coli was highly resistant to Ampicillin & highly sensitive to Gentamicin, Chloramphenicol, Streptomycin, Ceftriaxone &, Kanamycin. The study conducted by Bhandari P, Thapa G, *et al*, in 2015[12]found Escherichia Coli was highly resistant to amikacin and highly sensitive to Polymyxin B and Imipenem.

Surface Swab Sample

The most common isolate was Klebsiella Pneumoniae, whereas the study conducted by Jalapoor S, in 2011[14] found the most common was isolate Escherichia coli.

AST

The Klebsiella pneumoniae was highly sensitive to Gentamicin & Highly resistant to Tetracycline. Whereas, the study conducted by Jalapoor S, in 2011[14], found Klebsiella pneumoniae was highly sensitive to Gentamicin, Ampicillin, Chloramphenicol, and Ceftriaxone, and highly resistant to Co-Trimoxazole.

HAND Swab Sample

The most common isolate was CONS whereas in the study conducted by Chaka T E, *et al* in 2016[15] where the most common isolate was Staphylococcus aureus.

AST

The CONS was highly sensitive to Vancomycin. Whereas in the study conducted by Chaka T E *et al*. in 2016[15], CONS was highly sensitive to amoxicillin, clavulanic acid.

In this study, a total of 118 air samples & 179 swab samples were collected from different wards and OTs of the hospital, & 15 hand swab samples were collected from various laboratory professionals of the hospital. In this study, a total of 118 air samples & 179 swab samples were collected from different wards and OTs of the hospital, & 15 hand swab samples were collected from various laboratory professionals of the hospital.
For air samples, the maximum isolation percentage was from air samples of ICCU, Emergency Ward, Ortho dressing room, Psychiatry ward, Gyne. ward, TB ward, Orthopedic ward, Male & Female Surgery ward & ENT ward, and minimum isolation percentage were from air samples of Ophthalmology ward.

Gram-negative bacteria were more common than gram-positive bacteria. The overall most common isolate was Escherichia coli. The most common gram-positive bacteria isolated were Coagulase negative staphylococcus species followed by Staphylococcus aureus. All Staphylococcus aureus isolates were sensitive to Vancomycin & Erythromycin. All Coagulase-negative staphylococcus speciesisolate were sensitive to Vancomycin & Penicillin.

The most common gram-negative bacteria isolated were Escherichia coli followed by Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa. All Escherichia coli isolates were sensitive to tetracycline & Gentamicin. All Klebsiella pneumoniae isolate was sensitive to Co-trimoxazole & Gentamicin. All Proteus mirabilis isolate was resistant to tetracycline. Pseudomonas aeruginosa isolate was mostly resistant to tetracycline & Gentamicin.

For the swab sample, the maximum isolation percentage was from swab samples of the Ophthalmology ward, TB ward & Psychiatry ward and the minimum isolation percentage was from swab samples of MICU & high-risk labor room.

Gram-negative bacteria were more common than gram-positive bacteria. The overall most common isolate was Klebsiella pneumoniae. The most common Gram-positive bacteria isolated was Coagulase-negative Staphylococcus species followed by Staphylococcus aureus. All Staphylococcus aureus isolates were sensitive to Vancomycin & Erythromycin. All Coagulase-negative staphylococci species isolate were sensitive to Vancomycin. The most common Gram-negative bacteria isolate was Klebsiella pneumoniae followed by Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis & Proteus vulgaris. All Klebsiella pneumoniae and Escherichia coli isolate were sensitive to Gentamicin. Proteus mirabilis isolate was mostly sensitive to Co-trimoxazole. All Proteus vulgaris isolate were resistant to Tetracycline and all Pseudomonas aeruginosa isolate were resistant to Gentamicin. Maximum isolation percentage was from swab sample of warmer and minimum isolation percentage was from swab sample of Instrument Trolley, Laparoscopy Machine & X-Ray View Box.

For the hand swab sample, the most common isolate is Coagulase-negative staphylococci speciesGram-positive bacteria were more common than gram-negative bacteria. The most common gram-positive bacteria isolate was Coagulase-negative staphylococci species. All Coagulase-negative staphylococci species isolate were sensitive to Vancomycin. All Proteus vulgaris isolate were sensitive to Co-trimoxazole, Tetracycline & Gentamicin.

CONCLUSION

The isolation of pathogenic bacteria from the hospital environment in this study indicates that they can be vehicles for disease transmission. So, there is need for thorough disinfection and conscientious contact control procedures to minimize the spreads of these pathogens where interaction between patients, healthcare workers and caregivers is very common and frequent. It is also necessary to encourage the effective use of disposable hand gloves between patients and

to avoid touching fomites with gloved hands, which may be acting as source of infection.

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