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Original Research Article

Microbiological surveillance of hospital environment in tertiary care hospital in north India

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ABSTRACT

Background: Hospital acquired infection (nosocomial infection) are commonly occurring in developing as well as developed countries almost 50% of patients in developed countries and 75% in developing countries get infected inside the hospital to which they are not exposed earlier. The problem occurring in treatment to these patients is the resistance to certain antibiotics among these bacteria.
Methods: A swab soaked in nutrient broth was used to collect samples from the floor, walls, equipments, instruments, operation tables, etc. All the samples were labelled properly and immediately transported to the microbiology laboratory in Government medical college Jammu for processing.
Results: Out of 29 air samples positive is 9 and the percent is 31.0 swabs are 92. Out of 92 samples positive is 28 and the percent is 30.43. Out of 29 samples 3 samples are taken from orthopaedic OT there is no growth. 3 samples are taken from gynaecology OT no growth. 4 samples are taken from urology theatre 2 are *Staphylococcus aureus* and the percent is 50. 2 samples are taken from eye OT there is no growth.
Conclusions: These findings demonstrate that the microbiological quality of air and surfaces in operation theatre may be considered a mirror image of the hygienic conditions of the operating theatre.

Keywords: Nosocomial, Proteus, Bacterial, Air, Surveillance, Infection

INTRODUCTION

Nosocomial infections are infections that are acquired in the hospital or acute care setting in relation to the original condition of the patient. Infections are said to be hospital-acquired if they surface within 48 hours after admission to the hospital unit or within 30 days after the patient has been discharged home.[1]

In 1990 to 1996, the three most common gram-positive pathogens *S. aureus*, Coagulase-negative *Staphylococci*, and *Eenterococci* accounted for 34% of nosocomial infections, and the four most common gram-negative pathogens*; Escherichia coli, P. aeruginosa*, *Enterobacter spp* and *Klebsiella pneumonia* accounted for 32%.[2] Acquired antimicrobial resistance is the major anticipated problem in hospitals. VRE and MRSA are the major gram-positive pathogens of concern. *Pseudomonas aeruginosa*, *Klebsiella*, and *Acinetobacter* that harbor chromosomal or plasmid-mediated beta-lactamase enzymes are the major resistant gram-negative pathogens.[3]

They affect 1 in 10 patients admitted to hospital. Annually, this results in 5000 deaths with a cost to the National Health Service of a billion pounds. On average, a patient with hospital acquired infection spent 2.5-times longer in hospital, incurring additional costs of £3000 more than an uninfected patient.[4] Intensive care units (ICU) have the highest prevalence of hospital-acquired

infections in the hospital setting.[5] Nosocomial infections typically affect patients who are immunocompromised because of age, underlying diseases, or medical or surgical treatments.[6]

These comprise surveillance methods, prevention strategies and treatment programs. In the UK, Every Trust has infection control teams comprising an infection control doctor (usually a consultant microbiologist), an infection control nurse and a manager.[7,9] Surveillance is defined as "the ongoing, systematic collection, analysis, interpretation and evaluation of health data closely integrated with the timely dissemination of this data to those who need it (ref).There are two key aspects of surveillance systems: Surveillance is an organized and ongoing component of a program to improve a specific area of population health. Surveillance systems go beyond the collection of information; they involve mechanisms through which the knowledge gained through surveillance is delivered to those who can use it to direct resources where needed to improve health.[10]

Healthcare-associated infections (HAIs) are a common cause of morbidity and mortality in the United States and are among the most common adverse events in healthcare. Recently, new emphasis on HAIs as a patient safety and public health problem has underscored the need for systematic HAI surveillance as part of a broad-based prevention and control strategy Every day, HCAI results in prolonged hospital stays, long-term disability, and increased resistance of microorganisms to antimicrobials, massive additional costs for health systems, high costs for patients and their family, and unnecessary deaths.[11] Although HCAI is the most frequent adverse event in health care, its true global burden remains unknown because of the difficulty in gathering reliable data: most countries lack surveillance systems for HCAI, and those that do have them struggle with the complexity and the lack of uniformity of criteria for diagnosing it nosocomial infections are associated with a great deal of morbidity, mortality and increased financial burden. Intensive care is a risk factor for the emergence of antibiotic resistant bacteria. Gram-positive bacteria have overtaken Gram-negative organisms as the predominant cause of nocosomial infections.[12] Inadequate antibiotic therapy is associated with poor outcome and particularly with bacterial resistance. Infection control measures are important for the effective control, prevention and treatment of infection. Knowledge of emerging pathogens and resistance profile is essential for treatment against nocosomial infections. Shorter duration of treatment and correct dosage of antibiotic therapy is recommended to reduce the selection pressure for resistant isolates. Hand washing is the single most important measure to prevent nocosomial infections.[13]

METHOD

Streak method: this method is usually involved in the inoculation of samples in blood agar or MacConkey agar media. MacConkey agar is also called differential medium. It is also give us lactose fermenting colonies (LF) and Non lactose fermenting colonies (NLF). Blood agar is enriched media. It may be used for growing for number of bacteria but one on specific example is Streptococcus which requires blood for growth urology OT and surgical OT showed presence of microorganisms.[24,25] Chi Square test was applied to find significance of difference between the two. Values derived were: Correction=0.17, Degree of freedom=1 and P value=0.6874. Statistically not significant. Chi square test applied to find statistical significance of the differences observed in terms of total number of samples showing micro organisms: Chi square=3.47 and P value=0.6273. Statistically not significant. Blood agar: It is enriched media; bacteria lysing red cells show a clearing around their colonies.

Positive control: Staphylococcus aureus ATCC 25923. Final pH-7.3+0.2 (at 25°C). Mac-Conkey agar: It is a difficult media in which the inhibitory action of crystal violet on the growth of gram-positive organisms allows the isolation of gram-negative bacteria.[26] Positive control: *Escherichia coli* ATCC 25922. Final pH=7.2±0.2 (at 25°C). Negative control: ATCC25923. Principle: The exact mechanism of Gram reaction is not known. It may, however be attributed to:Gram positive bacteria have more acidic protoplasm, which may account for their retaining the basic primary dye more strongly than Gram-negative bacteria.

Biochemical tests

Catalase test principle: certain bacteria have an enzyme catalase which acts on hydrogen peroxide to release oxygen. Coagulase test principle: the bound coagulase is also known as clumping factor. It cross-links the α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual coccus sticks to each other and clumping is observed. Oxidase test: this test is perfume for the presence of electron transport system in pseudomonas species. Spot the filter paper with 10 to 20 ul of culture. Add 10 ul of freshly made oxidase reagent and look for production of a blue colour. Indole production test principle: The purpose of indole test is determining the ability of microbes to degrade the amino-acid tryptophan. Tryptophane is an essential amino acid that can undergo oxidation by way of the enzymatic activity of some bacteria. Conversion of tryptophan into metabolic product is mediated by the enzyme tryptophanase. Methyl red (MR test) principle: the purpose of this test detects the production of sufficient acid by fermentation of glucose so that pH of this medium falls and it is maintained below 4.5. Mannitol fermentation test: The purpose is to see if the microbe can ferment the carbohydrate (sugar) mannitol as a carbon source. Mannitol is fermented to produce acid end products, the pH of the medium will drop. A pH indicator in the medium changes color to indicate acid production. Urease test principle: This test detects the ability of an

organism to produce an enzyme urease which splits urea to ammonia. Ammonia makes the medium alkaline and thus phenol red indicator changes to pink/red in colour. Citrate utilization test principle: this test is used to study the ability of an organism to utilize citrate as the sole source of carbon for its growth. Liquid (Koser's) and solid (Simmon's) media containing citrate as a sole source of carbon can be used. Solid medium contains bromothymol blue as indicator. Citrate utilization results in alkaline pH that turns the indicator from green to blue. Sugar fermentation test principle: this test is to determine the ability of an organism to ferment a specific carbohydrate (sugar) incorporated in a medium producing acid or acid with gas. Muceller Hinton agar is used to check the sensitivity of micro-organisms to various antibiotics. Positive control: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. Final pH-7.3+0.2 (at 25°C). Antibiotic sensitivity testing*:* Some antibiotics are used to check the susceptibility of bacteria towards a particular drug, which is used in a treatment of patient and this test, is known as antibiotic sensitivity test. Antimicrobial sensitivity test are of different types: diffusion method, Kirby-Bauer disc diffusion method, stoke disc diffusion method, dilution method, broth dilution method, agar dilution method.

Kirby-Bauer disc diffusion method

Principle: the interpretation by zone size into susceptible (infection treatable with normal dosage, moderately susceptible (infection that may respond to therapy with higher dosage) or resistant (not treatable with the agent) is based on the interpretation chart.[27] Quality control organisms such as *E. coli* ATCC 25922, and S. aureus ATCC 25923 were tested periodically to validate the accuracy of the procedures.

RESULTS

During the period from 1 January to 30 June 2014, a total 142 samples was inoculated. The samples were taken from critical care areas and operation theatres in Govt. medical college and associated hospital Jammu. Out of 29 air samples positive is 9 and the % is 31.0 swabs are 92. Out of 92 samples positive is 28 and the % is 30.43. Control is Nil. Out of 29 samples 3 samples are taken from orthopaedic OT there is no growth. 3 samples are taken from gynecology OT no growth. 4 samples are taken from urology theatre 2 are staphylococcus aureus and the % is 50. 2samples are taken from eye OT there is no growth, 11 samples are taken from surgical OT 1 is CONS % is 9.09, 4 are *Staphhylococcus aureus* % is 36.36 and 1 is streptococcus % is 9.09. 4 samples are taken from CVTS 1 is staph. aureus % is 25, 1 sample are taken from neurology OT no growth and 1 sample are laprascopy OT no growth. The total number of air samples in different operation theatres was 29.

92 samples are taken from various critical care areas and operation theatres.

Table 1: Total number of samples 142. Air 29, Swabs 92 and 21 controls.

Type of sample	N	%
Air	29	20.42
Swabs	92	64.78
Control	21	14.78

Figure 1: Distribution of samples for positive and negative if any organism in critical care areas and operation theatres.

The organism are isolated are CONS, *Staphylococcus aureus sp, Streptococcus sp, Acinetobacter sp, Klebsiella sp, and pseudomonas sp* are isolated. The 8 swabs are taken from CCU.1 are *S. aureus* % is 12.5 another is *Pseudomonas* % is 12.5, 32 swabs are taken from ICU 1 is cons % is 3.12, 3 are *Staphylococcus aureus* % is 9.37, *Acinetobacter* are 2 % is 6.25, *Klebsiella* is 1% is 3.12, *Pseudomonas* is 1, % is 3.12. Burn ward 5 swabs are taken *S. aureus* 1, % is 20, *Klebsiella* 1, % is 20. 5 samples are taken from NICU *S. aureus* 1, % is 20 *Klebsiella* 1, % is 20.32 swabs are taken from surgical OT. CONS is 4 and the % 12.5, *S. aureus* 1, % is 3.12, *Acinetobacter* 3 and the % is 9.37, *Klebsiella* is 1, % is 3.12, *Streptococcus* 1, % is 3.12. Resucitation room 4 swabs are taken from it *S. aureus* % is 25, *Acinetobacter* is 1, % is 25. Labour room 6 samples are taken from it staph. aureus 1 5 is 16.66. The total number of swabs is 92 out of these 92 swabs 30 swabs are Wpl and 24 are Apl. Air samples are 29 out of these 29 samples 7 samples are Wpl and 9 samples are Apl. 21 samples are control 2 samples Wpl and Apl is nil.

DISCUSSION

Almost any pathogen can, on occasion, cause hospital infection but those are able to survive in the hospital environment for long periods and develop resistance to antibiotics and disinfectants are particularly important in this respect.[14] *S aureus* strains, resistant to multiple

antibiotics and belonging to phage type 80/81, spread globally in 1950sand 1960s, colonizing hospitals and causing nosocomial infection with such frequency that they came to be called hospital *Staphylococci*.[15]

Table 2: Various organisms isolated from air sample of various operation theatres. The organisms isolated are CONS, *Staphylococcus aureus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Pseudomonas* sp.

Name of OT	Total samples	Organism Isolated											
		CONS		*S. aureus* sp.		*A. bacter* sp.		*Klebsiella* sp.		*Pseudomonas* sp.		*Steptococcus* sp.	
		N	%	N	%	N	%	N	%	N	%	N	%
Orthopaedics	3	-	-	-	-	-	-	-	-	-	-	-	-
Gynecology	3	-	-	-	-	-	-	-	-	-	-	-	-
Urology	4	-	-	2	50%	-	-	-	-	-	-	-	-
Eye	2	-	-	-	-	-	-	-	-	-	-	-	-
Surgical	11	1	9.09	4	36.36	-	-	-	-	-	-	1	9.09
CVTS	4			1	25	-	-	-	-	-	-	-	-
Neurosurgery	1	-	-	-	-	-	-	-	-	-	-	-	-
Laprascopy	1	-	-	-	-	-	-	-	-	-	-	-	-

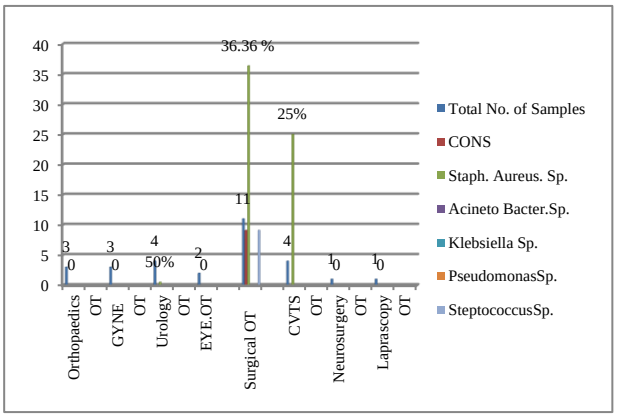

Figure 2: Various organisms isolated from air sample of various operation theatres. The organisms isolated are CONS, *Staphylococcus aureus* sp., *Streptococcus* sp., *Acinetobacter* sp., *Klebsiella* sp., *Pseudomonas* sp.

Table 3: Total samples that are oxicillin resistant S. aureus (MRSA).

Total air samples	Total oxicillin resistant S. aureus (MRSA) N (%)
29	7 (24.13)

The original phage type have since 111 but *Staphylococci* continue to be very common agent in hospital infection.[16] *Pseudomonas aerugionsa* and other pseudomonas species have always been important cause of hospital infection because of their intrinsic resistance to most antibiotics and ability to survive and even multiply at low temperature and in disinfectant solutions.[17] Bacterial contamination of operation and critical care areas like intensive care units in hospital setting had contributed significantly to high prevalence of nosocomial infection. In our study positive bacterial isolates were obtained (31%) from the air samples by open culture plate technique.[18,19] The effect of external factor can be explained by the presence of staphylococcus aureus isolates that predominant in sampled units by open plate method.

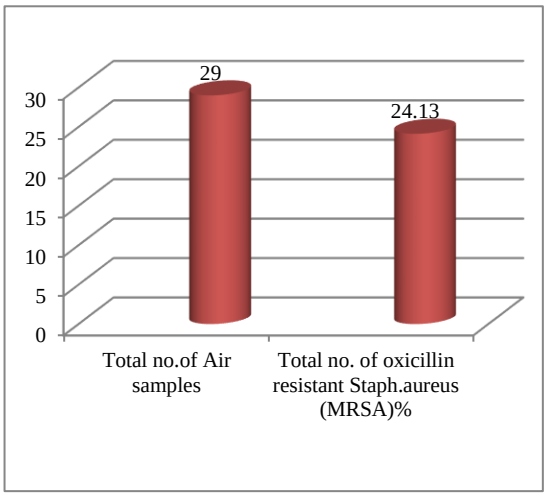

Figure 3: Total samples that are oxicillin resistant S. aureus (MRSA).

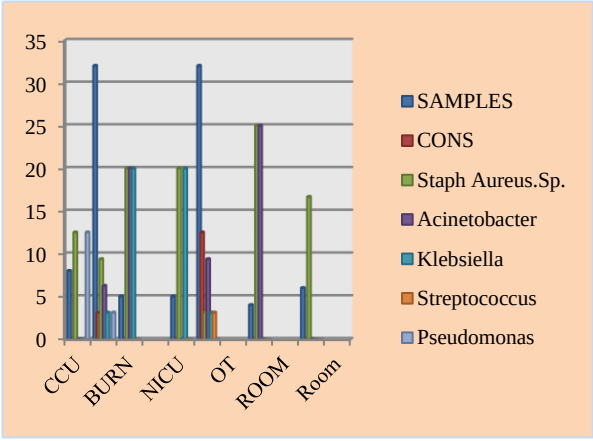

Figure 4: Different organisms are isolated from surface and articles of various critical care areas and operation theatres (n=92).

Table 4: Different organisms are isolated from surface and articles of various critical care areas and operation theatres (n=92).

Name of OT	Total samples	Organism isolated											
		CONS		*S. aureus* sp.		*Acinetobacter* sp.		*Klebsiella* sp.		*Streptococcus* sp.		*Pseudomonas* sp.	
		N	%	N	%	N	%	N	%	N	%	N	%
CCU	8	-	-	1	12.5	-	-	-	-	-	-	1	12.5
ICU	32	1	3.12	3	9.37	2	6.25	1	3.12	-	-	1	3.12
Burn ward	5	-	-	1	20	1	20	1	20	-	-	-	-
NICU	5	-	-	1	20	-	-	1	20	-	-	-	-
Surgical	32	4	12.5	1	3.12	3	9.37	1	3.12	1	3.12	-	-
RT room	4	-	-	1	25	1	25	-	-	-	-	-	-
Labour room	6	-	-	1	16.66	-	-	-	-	-	-	-	-

Table 6: The total number of surface/articles samples taken from different critical units and operation theatres, out of these 92 samples 8 samples are oxicillin resistant S. aureus (MRSA) (n=92).

Total samples	MRSA N (%)	Other N (%)
92	8 (8.69)	19 (20.65)

Table 7: The 92 swabs taken from different critical care areas and operation theatres and 29 air samples are taken from different operation theatres.

Samples	Total samples	WPL	APL
Swabs	92	30	24
Air	29	7	9
Control	21	2	0

Primarily *Staphylococcus aureus* is found in anterior nares, Axilla and groin and small number are carriers who can shed these bacteria in air (Hughes and Anderson, 1999). In contrast this level of bacterial contamination (16.66%) was recorded by swabbing method.[20] So the present study revealed that the higher bacterial contamination was found in air samples instead of surface or article sample.[21] In air sample as well as surface sample, the maximum growth of contaminated bacteria was observed in labour Room. The breakdown of bacterial pathogen shows *Staphylococci* predominant in all the units. Which is consistent with similar reported studies.[22] Microbiological quality of air may be considered as mirror of the hygienic condition of the operation theatres.[23] From the critical care units pseudomonas species was isolated from disinfectant solution from CCUs and ICUs. This study is fruitful for almost all population, who are frequently visiting hospitals.

The study gestures how you can prevent from hospital acquired infections also can bestow a gate way for various preventive precautions. The study can be more valuable if taken in great strength regarding sampling and broader covering areas of hospitals. Further studies can make this much clear if taken through a keen consideration.

CONCLUSION

These findings demonstrate that the microbiological quality of air and surfaces in operation theatre may be considered a mirror image of the hygienic conditions of the operating theatre. Open plate's method for air and swabbing technique for surfaces proved to be more valuable in detecting the contamination level in our setup with limited resources. Routine sampling of floor, walls or surfaces which are not in direct contact with patients are not the source of infection. They do not contribute in the prevention of nosocomial infection, unless there is an epidemic.

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