Article

S.Aureus Isolated from OTs & Antibiotic Susceptibility

PCR Based Identification of Staphylococcus. Aureus Isolated from Different Operation Theaters of Tertiary Care Hospital and Antibiotic Susceptibility by Disk Diffusion Method

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ABSTRACT

Objective: To isolate the presence of Staphylococcus. aureus from different operation theaters and confirmation by the help of PCR and to determine the antibiotic susceptibility pattern by disk diffusion method.
Study Design: Clinical Study
Place and Duration of Study: This study was conducted at the Institute of Microbiology and Biotechnology Department, University of Lahore, Pakistan from September 2016 to March, 2017.
Materials and Methods: To evaluate contamination in various operation theaters from Tertiary Care Hospital at Lahore city. Air contamination of operation theatre was evaluated by settle plate method. Petri plates containing media were opened on different places that include window, instrument table, and entrance and OT table for about 15 minutes. Total 12 media containing petri plates were opened at different levels in each operation theatre and incubated for 24 hours at 37c.Antibiotic susceptibility done by disk diffusion method and molecular identification was also done.
Results: To detect antimicrobial resistance, mecA and vanA gene were amplified and molecular identification of S.aureus was done by TStaG gene.14 out of 16 samples were positive for TStaGgene, 8/8 were positive for mecA and 0/7 for vanA gene.
Conclusion: S.aureus is commonly present in all operation theatres and causes a lot of lethal infections thus proper sterilization/disinfection and proper antibiotic selection is required for its treatment.
Key Words: Nosocomial infection, Operation theatre, Staphylococcus aureus, Hospital acquired infections.

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INTRODUCTION

The prevalence of nosocomial infections (NI)is increasing day by day and contamination of operation theatres (OT) is one of the important cause[1].In developing countries, these infections are involved in causing lethal problems. A study revealed that (HAI) ranges from 2.5 to 14.8%.[2]

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Multiple ways are involved in spreading contamination in operation theatres like unfiltered air, ventilation system, collection bags, drainage of wounds, indoor traffic, gown, transportation of patients, surgical team, gloves, inadequate sterilized instruments and foot wear.[3]

In Asia and Africa 40% of cases are due to NI. The most common pathogens involved in causing NI are S. aureus, S. epidermidis, Vancomycin resistant Enterococci, E.coli, Bacillus cereus, Klebsiella pneumonia and P. aeruginosa. The most important bacteria is S. aureus. From 1999 to 2005 the no. of cases admitted due to methicillin resistant Staph. aureus was 477927 that increased from 127036 to 278203. Another cause of bacteremia, cardiovascular infections and pneumonia isS.aureus.[4, 16]

Major cause of morbidity and mortality in hospitals all around the world is Methicillin Resistant S. aureus (MRSA). It is proved that environment is an essential part in developing resistance in microorganisms i.e. Staphylococcus survives in dry environment and can stick in clinical areas that are not cleaned properly.[5] The most effective antibiotic considered against

S.aureus is Vancomycin but now resistance is developing against it.[6]

There are number of PCR- based methods reported that are used for specific detection of these bacteria. Airborne bacteria in Operation Theater can be reduced upto 13- fold e.g. contamination through wound would reduce up to 50%. It all depends on proper disinfection, regular fumigation and proper disinfection of OT.[1, 20]

This research was designed to isolate the occurrence of staphylococcus aureus in Operation Theater environment and also check antibiotic sensitivity through disk diffusion method.

MATERIALS AND METHODS

This study was carried out at Institute of Microbiology and biotechnology Department, University of Lahore, Pakistan from September 2016 to March, 2017.

Sample Collection: Air contamination of operation theatre was evaluated by settle plate method. Petri plates containing media were opened on different places that include window, instrument table, and entrance and OT table for about 15 minutes. Total 12 media containing petri plates were opened at different levels in each operation theatre and incubated for 24 hour at 37c.

Isolation and Identification: For isolation and identification of bacteria colonies with different morphology were taken and carefully streaked on nutrient agar plates.

Microscopy and Gram Staining: Microscopy and Gram staining was performed for well isolated colonies on Nutrient agar, MacConkey,s agar and Blood agar.

Confirmation by Biochemical Tests: To differentiate between Staphylococci or Streptococci catalase and coagulase test were performed. All species of Staphylococcus were positive for catalase test and therefore differentiated from catalase negative Streptococcus species. S.aureus is coagulase positive.

Antibiotic Susceptibility Testing: Disk diffusion method was used to evaluate antimicrobial susceptibility of Staphylococcus aureus. Fresh Staphylococcus aureus colonies were inoculated in 5ml of normal saline and turbidity was compared with 0.5M MacFarl and standard solution. With the help of sterile cotton swabs inoculum was inoculated on Muller Hinton Agar plates, then discs were applied on it at left at 37º for 24 hours.

Molecular Identification: Molecular identification of S.aureus was done by TStaGgene amplification while to detect antimicrobial resistance against methicillin and vancomycin, mecA and vanA gene were amplified respectively.

RESULTS

Morphological and Biochemical Test: Staphylococcus aureus: is a gram +ve cocci with thick cell wall of peptidoglycan, facultative anaerobe, non-motile and non-spore forming bacteria. S. aureus produce circular pinhead convex yellowish colonies on nutrient agar, microscopically bunch of gram +ve cocci, produce bright yellow colonies on mannitol salt agar, produce beta hemolysis on blood agar, gave catalase and coagulase test positive. S. aureus occur as commensals on human skin and major pathogen cause nosocomial infection.

Molecular Identification of Isolated Bacteria: For molecular identification of S. aureus, 16 samples among 53 isolated S. aureus samples were picked for molecular identification. 14 out of 16 samples were amplified TStaG (370 bp) gene specific for S. aureus hence positive for this gene. For methicillin resistant S. aureusstrains mecA gene amplification was done and all 8 samples picked randomly were positive for mecA gene. While for vancomycin resistant strains of S. aureusvanA gene was amplified but none of the sample amplified vanA gene hence no vancomycin resistant S.aureus was present.

Table No. 1: Primer sequence for S.aureus identification, methicillin and vancomycin resistant genes

Sr. No.	Targeted gene.	5' to 3' primer sequence	References
1	TStaG422	F- GG. CC. GT. GT. TG. AA. CG. TG. GT. CA. AA. TC. A R- TT. AC. CA. TT. TC. AG. TA. CC. TT. CT. GG. TA. A	McClure ,J .M & Zhang, K., 2017)
2	mecA	F- AA. AA. TC. GA. TG. GT. AA. AG. GT. TG. G C R- AG. TT. CT. GG. AG. TA. CC. GG. AT. TT. G C	(Pournajaf et al., 2014)
3	vanA	F- AT. GA. AT. AG. AA. TA. AA. AG. TT. GC R- TC. AC. CC. CT. T T. AA. CG. CT. AA.TA	(Saadatet al., 2014).

Table No.2: PCR Conditions for S.aureus identification and antibiotic resistant genes

Sr. No.	Targeted gene(primer)	Initial denaturation	35 cycles of repeated			Final extension
			Denaturation	Annealing	Extension	
1	TStaG	95ºC 4 mint	95ºC 1 mint	57ºC 1 mint	72^0C 1 mint	72ºC 10 mint
2	mecA	95ºC 5 mint	94ºC 1 mint	55ºC 1 mint	72^0C 1 mint	72ºC 10 mint
3	vanA	98ºC 2 mint	98ºC 30 sec	48ºC 90 sec	72^0C 90 sec	72ºC 10 mint

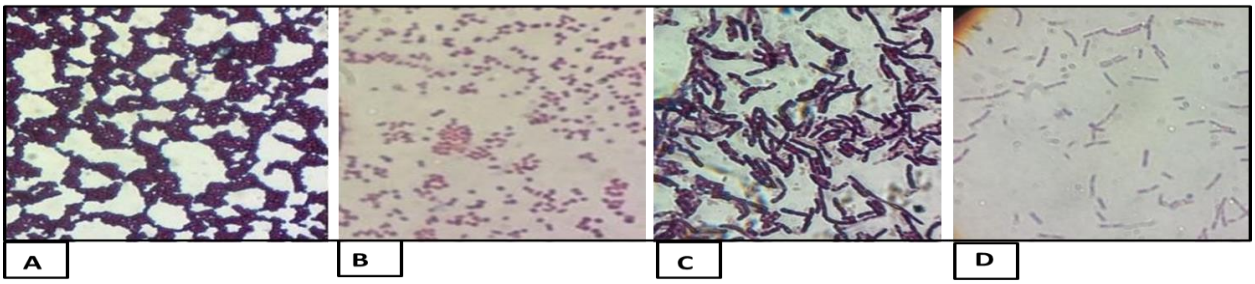
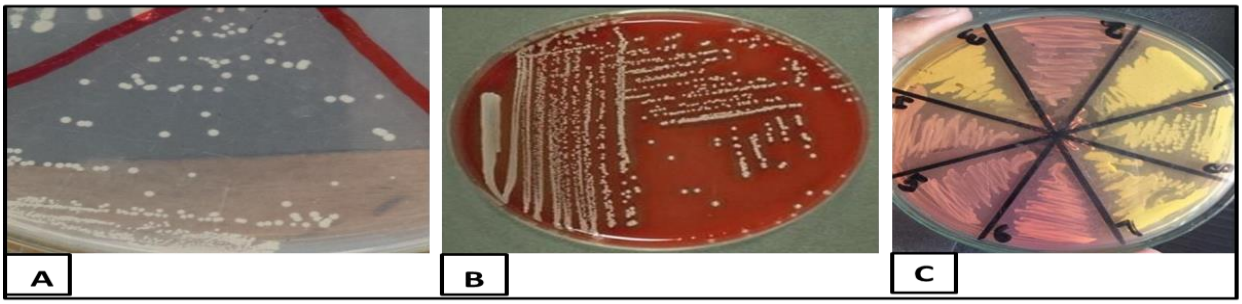


Figure No. 1(A): Gram positive cocci with bunch like appearance (Staphylococci) (B): Gram positive cocci bacteria in the form diplo cocci and tetrad (Micrococcus) (C): Gram positive rods bacteria(B.cereus) (D): Gram positive cocci bacteria in the form of short chains (Enterococci).



(A)Different growth morphologies on Nutrient agar (B) S.aureus showing ß hemolysis on blood agar.(C) S.aureus showing yellow growth on MSA

Antibiotic Sensitivity Test:

Figure No. 3(A): Vancomycin with reduced susceptibility (B) Methicillin sensitive(C) Vancomycin with reduced susceptibility and methicillin resistant.

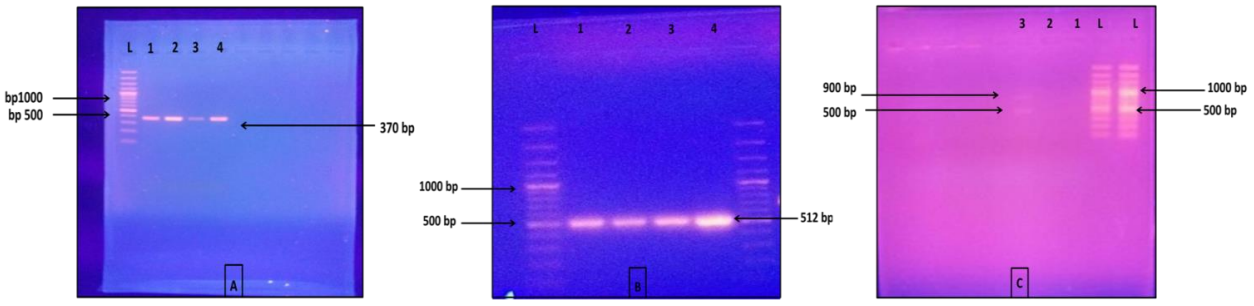


Figure (A) shows Agarose gel electrophoresis of PCR product using TStaG primer, L; DNA ladder 1000bp, lanes 1,2,3,4, DNA positive samples showing 370bp DNA bands. Figure B shows Agarose gel electrophoresis of PCR product using mecA primer, L: DNA ladder 1000bp, lanes 1,2,3,4, DNA positive samples showing 512bp DNA bands. Figure C shows Agarose gel electrophoresis of PCR product using vanA primer, L; DNA ladder 1000bp, lane 3 DNA positive sample showing 500 and 900 bp non-significant DNA bands

Table No.3 Primers used for PCR

Sr. No.	Targeted gene of Staphylococcus aureus	Amplicon size (bp)	Positive samples
1	StaG	370 bp	14/16
2	mecA	533 bp	/8
3	vanA	032 bp	0/7

DISCUSSION

Nosocomial infections caused by different microbes (commonly bacteria) are a real threat in developing nations where hygienic conditions are poor. More than half of the nosocomial infections are due to bacterial contamination i.e. bacteria present on skin, clothes, aerosol drops can contaminate OT air and also deposit on surfaces[15]. Diseased carrier patients and dirty wounds also contaminate operation theatres.[8] Bacterial agents commonly responsible for nosocomial infections include S.aureus, Streptococcus species, CoNS, P.aeruginosa, B. cereus, Enterococci, E. colietc[9]. This study was designed for the evaluation of contamination atdiverse or various Operation Theaters of a tertiary care from Lahore city. Evaluation was done by using settle plate method at different places of OTs such as entrance, OT table, window and instrument table. Microbial contamination of five different operation theater of a tertiary care hospital was checked. Among the isolated bacteria gram positive cocci i.e Staphylococcus aureus was identified in high percentage. S. aureus is a nosocomial causative pathogen, emergence of vancomycin (effective drug for S.aureus) resistant strain in different part of world is challenging.[10,18] S.aureus amplified for TStaGgene specific for S. aureus. 13 out of 15 randomly selected samples were positive for the TStaG gene, producing 370bp product. Similar result was observed in previous studies of [7, 19], hence confirming S. aureus. For detection of MRSA strain mecA gene was amplified for 8 isolates. All S. aureus strains was positive for mecA gene producing 533 bp band as observed in previous study.[11,17]Not a single MRSA strain amplified vanA resistance gene for vancomycin hence confirming absence of vancomycin resistance strain among MRSA. Although there is reports of reduced susceptibility and resistance to vancomycin but in present study it was confirmed that no strain possesses vancomycin resistant vanA gene. Antibiotic resistance of S. aureus by Disk Diffusion Method against different antibiotics was as follow: for Levofloxacin 4/53 (7.55%), for Ceftriaxone 2/53 (3.8%) for Azithromycin 15/53 (28.3%), Amoxicillin/clavulanate 18/53 (34%), for Methicillin 40/53 (75.5%) and Vancomycin 11/53 (20.75%). In present study antibiotic susceptibility showed that Ceftriaxone and levofloxacin are the most effective drugs with least resistance percentages of 3.8 and 7.5 respectively, these finding also observed in this study.[12,13]It is more than 75% isolates was Methicillin resistant also confirmed by 46 PCR detection of mecA gene. Although percentage of methicillin resistance S. aureus is different around the globe but in previous study [14]that percentage of around 55% was observed and also present that MRSA strains was sensitive to vancomycin. Vancomycin resistant MRSA are real challenge in wait but in present study all Methicillin resistant isolates are sensitive to vancomycin. Amplification of vanA gene also confirmed the susceptibility test as not a single isolates produce require band of 1032. Instead non-specific bands of size between 500- 900bp might be indicating emergence of resistance.

CONCLUSION

Nosocomial infection can be reduced by proper sterilization of instruments and fumigation of operation theatre which can kill the deadly bacteria i.e S.aureus. This study showed that the vancomycin resistant gene is so far not present and S.aureus can be treated with vancomycin. So selection of proper antibiotic and cleanliness of operation theatre is very important.

Author's Contribution:

Concept & Design of Study:	Noreen Sarwar
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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