ORIGINAL ARTICPLE

Isolation of Gram Positive and Gram Negative Bacteria from operation theaters of Tertiary Care Hospital and Molecular Detection of *Staphylococcus Aureus Genes*

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ABSTRACT

Aim: To identify the microorganisms in different operation theaters in tertiary care hospital and characterization of isolates on the basis of biochemical test and molecular level.
Methodology: Microbial contamination of five different operation theaters were selected including General surgery (OT1), gynecology (OT2), urology (OT3), orthopedics (OT4) and cardiac (OT5). Air samples of five different operation theatres were collected by Settle Plate Method. Sterile plates of nutrient, blood and Macconkey's agar were kept open for 15 min at four different places in operation theatres for air sampling.
Result: Staph. aureus was commonly found in OT's and to confirm this bacteria, molecular identification was done by amplifying TStaG gene.
Conclusion: Microbial burden is too much high in operation theatres and proper sterilization and fumigation is required.
Keywords: Hospital Acquired Infection, Operation Theatre, Staphylococcus aureus

INTRODUCTION

Nosocomial Infections are life-threatening problem in developing countries. A survey revealed that rate of Hospital Acquired Infection (HAI) ranges from 2.5 to 14.8%.[1] Surgeries play a major role in preventing death and disabilities for those who are suffering from tumors, obstetrical complications, injuries, emergency abdominal or non-abdominal conditions which can affect the quality of life i.e. cataract.[2] Successful surgeries improve the quality of life and prevent transmission of infectious diseases like Hepatitis, AIDS. Surgical conditions usually put the people out of work and put them into poverty i.e. a survey in Pakistan, blindness due to cataract was associated with poverty.[3] Such type of infections are called nosocomial infections. These infections are not related to the original disease, which brings patient to the hospital.[4] Operation theatre contamination is severely life threatening for the Transplantation, Cardiac, Neurosurgeries, Bladder and Tumor operations[5].

METHODOLOGY

Bacterial contamination in operation theatre air was evaluated by settle plate method. Petri plates containing sterile media i.e. Nutrient agar, Blood agar, MacConkeys agar were opened at four different places (entrance, OT table, window and instrument table) for 15 minutes. 12 plates were opened in each operation theatre at different levels and incubation for 24 hours at 37ºC. When incubation is done, colonies with different morphology were carefully streaked on fresh nutrient agar plates. Gram staining was done for well-isolated colonies on nutrient agar, blood agar and MacConkeys agar. Spore staining was performed for gram positive rods.

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Biochemical properties: Bacteria that appeared as Staphylococci or Streptococci were further tested for catalase and coagulase test. All Staph. species were catalase positive hence were differentiated from catalase negative Strept. species. Micrococcus species found in the form of tetrad or diplococcus. Catalase test is performed for Micrococcus species. Coagulase test is specifically performed to identify S. aureus and grown on Mannitol Salt Agar (MSA).

DNA Extraction of S. Aureus: DNA extraction was done by chemical method. Isolated colonies of S. aureus were cultured in LB broth and incubated for 24 hr at 37ºC. Now 1.5ml of cultured broth poured into sterile eppendorf and performed Isopropanol method and gets DNA and then stored at -20ºC for further used.

RESULTS

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. aureus strains, mecA gene amplification was done and all 8 samples picked among S. aureus confirmed by TStaG gene amplification were positive for methicillin resistance gene as well. While for vancomycin resistant strains of S. aureus vanA gene was amplified for TStaG positive S. aurues isolates but none of the sample amplified vanA gene. Hence no vancomycin resistant S.aureus was present. B.cereus confirmed by 16srRNA gene amplification. Out of 7 bacteria

for Vancomycin resistant gene, one bacteria showed bands in PCR product of 500 and 900bp size. Required band was 1032 but it showed at 900 and 500bp size in the table.

Operation Theater 1 (General Surgery): Total 71 bacteria were isolated from OT-1 at different level and among them, %age of S.aureus were 22.5%, CoNS species 10.9% ,Micrococcus species 8.8%, Enterococcus species 9.3%, Bacillus .cereus 13.2%, other bacilli species 9.8%, listeria monocytogenes 9.4%, E. coli 2.8 % and Pseudomonas .aeruginosa13.4%.

Operation Theater 2 (gynecology): Total 61 bacteria were isolated from OT-2 at different level and among them %age of S. aureus were 19.5 %, CoNS species 11.4%, Micrococcus species 10.4%, Enterococcus species 10.2%, Bacillus.cereus 9.3%, Other bacilli 14.8%, listeria monocytogenes 6.5%, E.coli 7.8% and P.aeruginosa 10.2%.

Operation Theater 3 (Urology): 50 bacteria were isolated from OT-3 at different level and among them %age of S.aureus were 18.1%, CoNS species 11.4%, Micrococcus species 12.1%, Enterococcus species 16.9% Bacillus .cereus 16.6%, Other bacilli species 10 % and E.coli 14.2%.

Operation Theater 4 (Ortho): Total 42 bacteria were isolated from OT-4 at different level and among them %age of S. aureus were 20.5%, CoNS species 13.4%, Micrococcus species 6.5%, Enterococcus species 8.6%, B. cereus 22.6%, Other bacilli species 17.8%, E. coli 2.9%, Streptococcus species 7.7%.

Operation Theater 5 (Cardiac): Total 31 bacteria were isolated from OT-5 at different level and among them %age of S.aureus were 16.5% CoNS 9.8%, Micrococcus 15.4%, B. cereus 18.9%, Other bacilli 22.3% E.coli 1.8%, P.aeruginosa 15.4%.

Morphological and Biochemical tests: Fig 1, 2 and 3 show morphological changes and biochemical tests of S. Aureus.

Fig 1:
(A): Gram positive cocci in the form of chains (Streptococci)
(B): Small pinpoint colonies of staph aureus
(C): Spore staining of gram +ve rods by malachite green
(D): Gram –ve rods showing pink color by Gram staining.

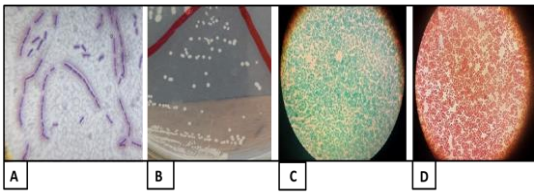

Fig 2:
(A)Different growth morphologies on Nutrient agar
(B) S.aureus showing ß hemolysis on blood agar.
(C) S.aureus showing yellow growth on MSA

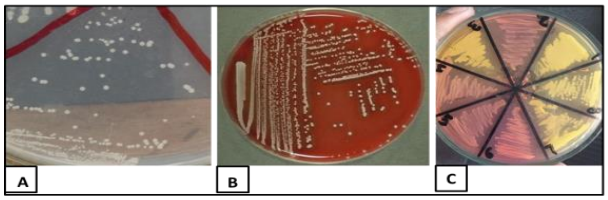

Fig 3:
(A) S.aureus showing coagulase test +ve by clump formation.
(B):Bubble formation showing +ve catalase test

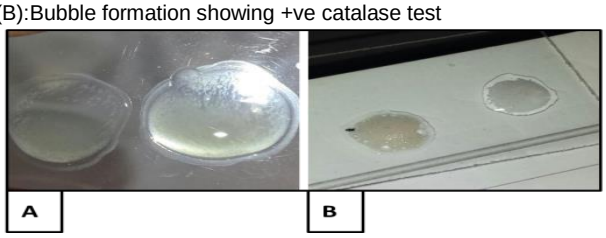

Table 1: Primer sequence for S. Aureus identification, methicillin and vancomycin resistant genes

Targeted gene.	5' to 3' primer sequence	References
TStaG422	F- GG. CC. GT. GT. TG. AA. CG. TG. GT. CA. A A. TC. A R- TT. AC. CA. TT. TC. AG. TA. CC. TT. CT. G G. TA.A	(McClure & Zhang, 2017)
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)
vanA	F- AT. GA. AT. AG. AA. TA. AA. AG. TT.GC R- TC. AC. CC. CT. T T. AA. CG. CT. AA.TA	(Saadat et al., 2014).

Table 2: Primers used for PCR

Targeted gene of Staph. aureus	Amplicon size (bp)	+ve samples
TStaG	370 bp	14/16
mecA	533 bp	8/8
vanA	1032 bp	0/7

DISCUSSION

The evaluation was done by using settle plate method at different level of OTs. Among the isolated bacteria gram positive cocci i.e. Staph aureus, CoNS species and Micrococcus species were the common, isolated from every OT. From above mentioned results it is obvious that gram positive cocci was the leading contaminant of OT environment as almost 55% of isolated samples were of gram positive cocci in all 5 OTs. Among gram positive cocci, S. aureus was the leading contaminant of OT environment in individual OT (≥ 16.46% and ≤22.46%) as well as collectively in all OTs (19.4%). Although in number of previous studies[6]. S. aureus was reported but in what is unusual in present study was the fact that pathogenic S. aureus was the leading contaminant of OT air. Coagulase negative Staphylococci were second leading contaminant of OT environment among gram positive cocci with

percentage between (≥9.8% and ≤13.4%) at individual OT level and well as an overall percentage of 11.4%. Similar kind of results was observed in the studies[7]. Other gram positive cocci isolated were micrococcus, enterococcus and streptococci species. In present study streptococci was only isolated specie from orthopedic OT and is in accordance with findings where Streptococci was isolated from a few OTs i-e from surgery and eye OT. Enterococci and micrococci species were isolated from almost all OTs[6].

For the Molecular identification of vancomycin resistant S.aureus strain genus specific gene TStaG of S. aureus was amplified. 14 out of 16 randomly selected samples were positive for the TStaG gene, producing 370bp product. Similar result was observed in previous studies of hence confirming S. aureus. For detection of MRSA strain mecA gene was amplified for 8 S. aureus isolates. All 8 S. aureusstrains were positive for mecA gene producing 533 bp band as observed in previous study[8].

CONCLUSION

S.aureus is major pathogen isolated and it is involved in skin, systemic infections and surgical site infections. Vancomycin was the most effective drug but now showing intermediate resistance which is real threat.

REFERENCES

1. Ahoyo TA, Bankole HS, Adeoti FM et al. 2014. Prevalence of Nosocomial Infections and Anti-infective Therapy in Benin: Results of the First Nationwide Survey in 2012. Antimicrobial Resistance and Infection Control 3:17
2. Debas TH, Gosselin R, McCord C et al. 2006. Disease Control Priorities in Developing Countries 2nd edition. Chap 67 Surgeries. Bookshel
3. PLoS Medicine Editors, "A Crucial Role for Surgery in Reaching the UN Millennium Development Goals." PLoS Medicine. 5.8 (2008): 1165-1167. Accessed on 29 October 2010.
4. Revelas A. 2012.Healthcare associated infections: A public health problem. Niger Med J. 2012 Apr-Jun; 53(2): 59–64.
5. Ensayef S, Al-shalchi S, Sabbar M. 2009. Microbial Contamination in the Operating Theatre: A Study in a Hospital in Baghdad. Eastern Mediterranean Health Journal, Vol. 15, No.1, 2009 219.
6. Edmiston CE, Seabrook GR, Cambria RA et al. 2005.Molecular Epidemiology Of Microbial Contamination In The Operating Room Environment: Is There A Risk For Infection? doi:10.1016/j.surg.06.045
7. Javed I, Hafeez R, Zubair M et al. 2008. Microbiological Surveillance of Operation Theatres and ICUs of A Tertiary Care Hospital, Lahore. Biomedica Vol. 24
8. Pournajaf A, Ardebili A, Goudarzi L et al. 2014. PCR based identification of Methicillin resistant Staphylococcus aureus strains and their antibiotic resistance profiles.Asian Pacific Journal Of Tropical biomedicine. vol 4. 293-297.