Molecular Detection of Some Virulence Genes in Stahpylococcusaureus Isolated from Different Human Clinical Specimens

Khaleid Yassen AL-Zamily[1], Niran Kadhim F. AL-Rubaey[2], Jundi Alak Mahdi Al-Buhilal[3]

[1]Assist Prof. Dr. Department of Medical Laboratories Techniques, Kut Technical Institute, Middle Technical University, Baghdad, Iraq, [2]Lect. Dr. Department of Medical Education, Hammurabi College of Medicine, University of Babylon, Babylon, Iraq, [3]Assit prof. Dr. Institute of Medical Technology Al-Mansour,Middle Technical University, Baghdad, Iraq

Abstract

Staphylococcus aureus is a significantbacteria in numerous simple and more severe diseases of humans because it possessesmanyvirulence factors which controlled byvarious genes that promoteinvention of the host cells.

A total of Ninetyisolates of *Staphylococcus aureus* obtained from various human clinical specimens distributed asurine samples (30/90) (33.3%), wound swabs (28/90) (31.1%), blood samples (24/90) (26.7%) and sputum swabs (8/90) (8.9%). The clinical specimens were submitted to diagnostic microbiology laboratories of Al-Hillah Teaching Hospital,Babylon maternity and children's Hospital and AL-Mahaweel General Hospital during the interval from April to September 2019, in Babylon Governorate, Iraq.

The multiplex PCR assay was achieved to identify Fourselected virulence genes such as (*sea*, *seb, eta* and *tst*) in *S. aureus* strains by using specific primers and depending from sizes of the products PCR amplicons. Each90 isolates of *S.aureus* in human clinicalspecimens were examined.T he results revealed that,the most frequent gene is *sea* 74 (82.2%), followed by *seb* gene 71(78.9%), *tst*gene 44 (48.9%) and *eta*gene 32 (35.6%) was reported as the least frequent detected gene.

Furthermore, it was found that, the predominancetoxin genes represent the highestrate in *S.aureus* strains isolated from wound swabs 85 (38.5%), followed by bloodsamples 69(31.2%), urine samples 54(24.4%) and finally the sputum swabs 13 (5.9%).

Keywords: *: Staphylococcus aureus, virulence genes, molecular detection, enterotoxine.*

Introduction

Staphylococcusaureus is a gram-positive, coccalbacterium, responsible for both nosocomial and community-acquired infections and implicated in hospital acquired infections. It causes a different illnesses extending fromsoft tissue to skin infections to severe life-threatening infections such as toxic shock syndrome (types 1 and 2)[1]. *S. aureus* is implicated in various diseases like impetigo, furuncles or boils, cellulitis and postoperative wound infections at different destinations. The organism is associated with serious and life-threatening infections including osteomyelitis, pneumonia, bacteremia, cerebritis, meningitis, acute endocarditis, myocarditis and pericarditis. Furthermore, it is associated with toxin-

Corresponding Author:
Dr. Niran Kadhim F. AL-Rubaey
Lect. Dr. Department of Medical Education,
Hammurabi College of Medicine, University of Babylon, Babylon, Iraq
e-mail: dr.nirranfarhood@yahoo.com
Mobile: 009647800406700

related diseases [2]. Additionally, it is widely prevalent and related withurinary tract infections [3]. *S. aureus* is capable of producing a series of virulence factors such as surface associated adhesions, enzymes and exotoxins, which play the main role in its invasive potential and pathogenicity [4]. Some of the virulence factors, such ashemolysins (Hlα and Hlβ), fibronectin-binding proteins A and B, Panton-Valentine leucocidin (PVL), enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) [5], coagulase and exfoliative toxin (ET) [6]. There are six serotypes of staphylococcal enterotoxin have been recently characterized, the most widely recognized groups are *Sea*,*Seb*, *Sec*, *Sed* and See[7,8]. These groups are the essentialcausative agent of food poisoning in humans and animals and this can result in intense intestinal peristalsis [9]. The superantigens group, including SE, TSST and ET toxins, these exotoxins display proteolytic and toxic or lytic effectiveness in the cells that assist local invasion andspread. Moreover, it has been reported that ETs ETA and ETB are either in coupling or separated participatory in the appearance of staphylococcal scalded-skin syndrome [10]. The gene of some toxins is situated in the chromosomal DNA, whereas, the others might be situated in mobile and transferable extra chromosomal DNA and by horizontal gene transfer (HGT) that is able translocating between bacteria [11]. Genes for staphylococcal enterotoxin groups,for example, *sea* geneis harbored by a bacteriophage vector, *seb* and *sec* genes are situated on the chromosomes, while *sed* geneisconveyed by a plasmid (pIB485) [12].

The current study was aimed to estimate the frequency, detect the existence of some selected virulence genes such as *(sea*, *seb, eta* and *tst*) in *Staphylococcusaureus* strains isolated from different human clinical specimens and evaluated the relationships between these clinical specimens and their abilityto yield virulence factors.

Materials and Method

Isolates Collected: All the isolates analyzed in this study,90 non duplicates *Staphylococcusaureus* isolates were cultured collections from various clinical specimens of patients suffering from different systemic infectionsdistributed as urine samples (30), wound swabs (28), blood samples (24) and sputum swabs (8). These clinical specimens were submitted to diagnostic microbiology laboratories of Al-Hillah Teaching Hospital, Babylon maternity and children's Hospital and AL-Mahaweel General Hospital during the interval from April to September 2019, in Babylon Governorate, Iraq. The all demographic data such as type of infection, site of specimen isolation, age and gender of patients were listed in the diagnostic laboratories.

Laboratory Diagnosis:

Bacterial Isolation and identification: According to the procedures recommended by[13], Confirmation the all of isolates as *S. aureus*was based on the inoculated onto mannitol salt agar and incubated reng 24-48 HR. in at 37°C. Colonies was identifiedrelied upon the colony size, shape, color, borders and texture. Colonies showing the common morphological appearance were investigated as *S. aureus* by Gram staining, then specimens were exposed to the Biochemical tests such as (Coagulase, Catalase, Citrateutilization, Urase, Nitrate reduction and Vogas-Proskaurtests).After concurrence, the samples was put in the medium TSB which includeing 20% at -20º C of glycerol until further usage.

The detection molecular of genes virulence in Staphylococcus aureus: The detection of Four virulence genes (*sea*, *seb, eta* and *tst*) of the *S. aureus* isolates was done as previously described by using multiplex PCR[6]. itemized sequences of the initial was summarized in Table (1).

Table (1): Oligonucleotide primer sequence and size amplicon

Target gene	Initial Sequence from 5′ to 3′	Product size (bp)	Reference
sea	GSEAR-1GGTTATCAATGTGCGGGTGG GSEAR-2CGGCACTTTTTTCTCTTCGG	102	Mehrotra*et al.,* 2000
seb	GSEBR-1 GTATGGTGGTGTAACTGAGC GSEBR-2 CCAAATAGTGACGAGTTAGG	164	
eta	GETAR-1 GCAGGTGTTGATTTAGCATT GETAR-2 AGATGTCCCTATTTTTGCTG	93	
tst	GTSSTR-1 ACCCCTGTTCCCTTATCATC GTSSTR-2 TTTTCAGTATTTGTAACGCC	326	

Multiplex PCR conditions: Bacterial DNA was improvedthroughutilizerprimer pairs for each gene and were prepared by using the master mixes of components from the GeneAmp kit (Perkin-Elmer, Norwalk, Conn.) and according to manufacturer'sinstructions with some modifications.Themixture reaction the volume final completedto50µl with sterilized D.W. Multiplex initialcontained 200 µMdeoxy-nucleoside tri-phosphates, 5 µl of 10× buffer reaction, 1.5 mM of $MgCl_2$,20 pmol each of *sea* and *seb* primers, 50 pmolof *eta*, 20 pmolof the *test*. 2.5 U of *Taq* DNA polymerase(AmpliTaq DNA polymerase, Perkin-Elmer) and 10 - 1,000 ng of template DNA.

Conditions of the Thermal cycling: Conditions of the Thermal cycling was performed according of the following in table (2).

Table (2): multiplex PCR for detection of sea, seb, eta and tstgenes virulence in Staphylococcus aureus strains

Steps	Temperature (°C)	Time (min)	No. of cycles
Initial denaturation	94	5	1
Denaturation	94	2	35
Annealing	57	2	
Extension	72	1	
Final extension	72	7	1

The PCR products and 100bp molecular weight DNA ladder were isolated by electrophoresis. The electric flow was permitted at90 V for 30 min. Detection was acknowledged by the existence of a specific DNA bands on agarose gel 1.5 %, stained with bromide ethidium dye and finally the bands were visualized on UV transilluminator and photographed via utilizer photo documentation method. The results positive was distinquished when the DNA band equal to the aim size product .

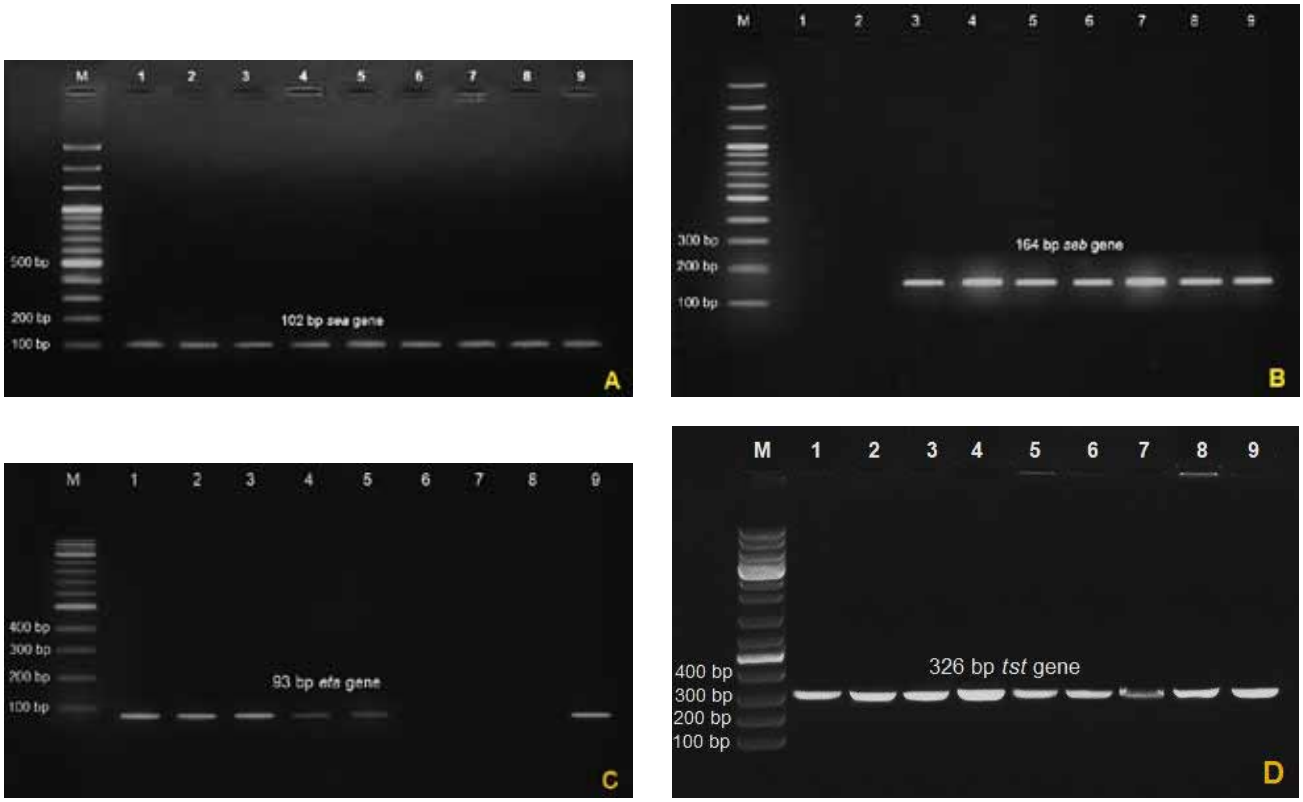
Results

Ninetyisolates of *Staphylococcus aureus* wascultured collections from various clinical specimens of patients suffering from different systemic infectionsdistributed asurine samples (30/90) (33.3%), wound swabs (28/90) (31.1%), blood samples (24/90) (26.7%) and sputum swabs (8/90) (8.9%), the results appear in table (3).

Table (3): Distribution of Staphylococcus aureusisolates according to the type of specimens

Type of specimens	No. of positive specimens	Percentage
Urine samples	30	33.3 %
Wound swabs	28	31.1 %
Bloodsamples	24	26.7 %
Sputum swabs	8	8.9 %
Total	90	100 %

The all (90) isolates of *S. aureus* were subjected to the multiplex PCR assay to identify Four virulence genes (*sea*, *seb, eta* and *tst*) by using specific primers and rely of sizes products amplicon of the PCR, results of PCR are shown in fig. (1).



Figures (1) Agarose gel electrophoresis of multiplex PCR assay to identify Four virulence genes (sea, seb, eta and tst) in Staphylococcus aureus strains isolated from wound, blood, urine and sputum specimens. Lane M: marker with 100 bp ladder.(A) Lanes 1-9: positive to sea gene, product size: 102 bp (B)Lanes 1 and 2:negative to seb gene, product size: 164 bp, while lanes 3-9: positive to seb gene. (C) Lanes 1-5 and 9: positive to eta gene, product size: 93 bp, while lanes 6-8: negative to eta gene. (D) Lanes 1-9: positive to tst gene, product size: 326 bp.

The results found that, thesea gene were the utmost frequent 74 (82.2%), followed via seb 71 (78.9%), tst 44 (48.9%) and *eta* 32 (35.6%) reported as the least frequent detected gene.

On the other hand, The results revealed that the prevalence of the virulence genes in*S. aureus* strains represent the highestrate in wound swabs85 (38.5%), followed bybloodsamples69 (31.2%), urine samples54 (24.4%) and sputum swabs13 (5.9%). The results are shown in the table (4) and the prevalance of (*seb, sea, eta* and *tst*) genes in*S. aureus* isolates regarding the clinical specimens of different infections were shown in figure (2).

Table (4): Distribution of genes virulence in Staphylococcus aureus isolates from clinical specimens of different infections

Type of specimen	sea %	seb %	tst %	eta %	Total %
Wound swabs	27(30%)	26 (28.9%)	18(20%)	14(15.6%)	85 (38.5%)
Blood samples	22 (24.4%)	21 (23.3%)	16(17.8%)	10(11.1%)	69 (31.2%)
Urine samples	20(22.2%)	19 (21.1%)	8(8.9%)	7 (7.8%)	54(24.4%)
Sputum swabs	5(5.6%)	5 (5.6%)	2(2.2%)	1(1.1%)	13(5.9%)
Total	**74(82.2%)**	**71(78.9%)**	**44 (48.9%)**	**32(35.6%)**	**221(100%)**

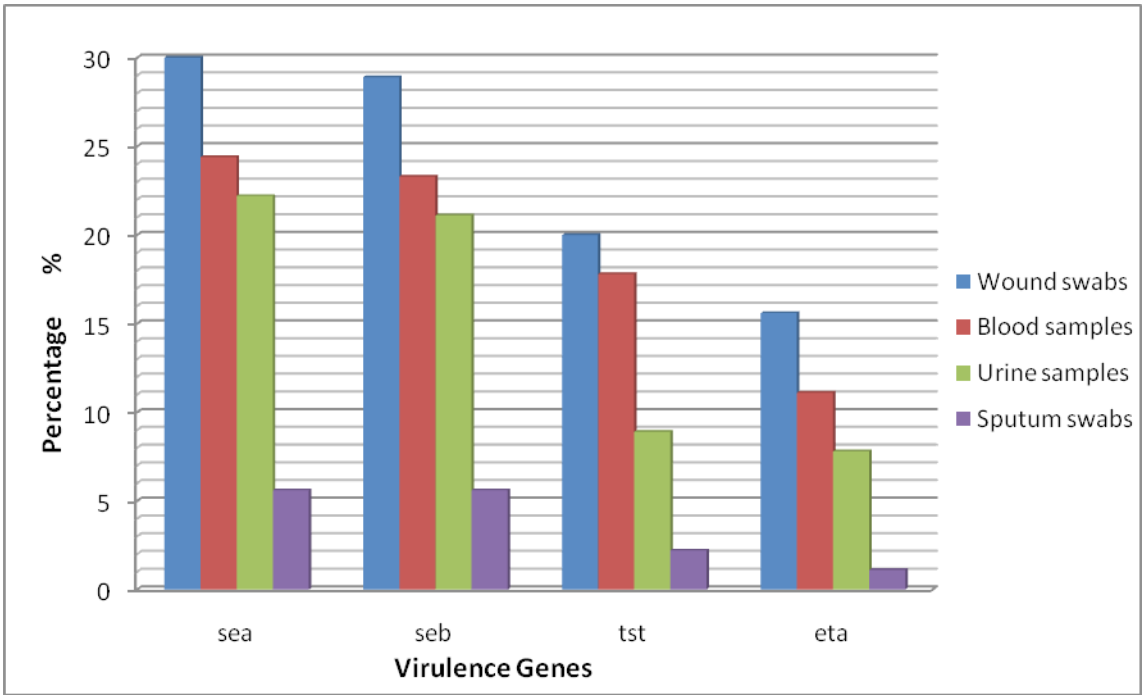


Figure (2): Prevalence the genes virulence in Staphylococcus aureus isolates for clinical specimens of several infections

Discussion

*Staphylococcusaureus*is a significant pathogen that contributed to the severity of the infection in humans because of its producing various virulence factors that are involved in colonization and the invention of the host leading tocause subsequent infections, these virulence factors are linked with various virulence genes.

In this study, the findingsrevealed that the *sea* gene was the most prevalent at a rate of (82.2%) followed by the *seb*(78.9%), *tst* (48.9%), while the *eta* gene was the leastfrequently detected gene at a rate of (35.6%). The high prevalence of *sea* gene refers to it plays animportant role in the pathogenesis of *S. aureus* infection.These results were agreed to a previous result studies which indicated that *sea* gene was the most prevalent(40.6%) followed by *seb, tst a*nd *eta* genes (19.6%, 12.8% and 11.3%) respectively in*S. aureus* isolated from different patients[14]. Different other previous study revealed by [15]demonstrated that the*seb* gene were the utmost prevalent (44.3%) followed by the *sea* gene (32%), while *tst* gene was identified at a very low rate (1%) among *S.aureus* strains in various kinds of disease, also pointed out that the pathogenicity of *S. aureus* is related to its ability forantibioticresistance and the toxin production. However, the result of our study is in contrast with the results of the study which indicated that *sea* gene were the utmost frequent gene (33%) and *seb* gene was identified at a very low rate (5%), while the *tst* genes was not identified in any isolates [16].Additionally,the low rate of *eta* gene in the current study is in accordance with previous study in Baghdad, Iraq, reported by [17],who found that *eta* gene was detected at a lower rate in *S. aureus* isolates from various clinical specimens and the result is in agreement with theother resultreported in Cote D'Ivoire [18]. Exfoliative toxins were caused impetigo consider as as one of the fundamental bacterial diseases [19].

Moreover, the results indicated that the majority of toxin genes produced by *S. aureus* strains were isolated from wound swabs at a rate of (38.5%), followed by blood samples (31.2%), urine samples (24.4%) and finally sputum swabs (5.9%).

S. aureus strains produce enterotoxins related with the food poisoning have antigenic and emetic effectiveness [20].Thus, we believe that the presence of enterotoxin genes in the strains isolated from wounds, blood, urine and sputum specimens of different infections is probably due to human or environmental

contamination via the existence of open injuries. These results were correspondedto the results study conducted in South-Western Nigeria by [21], who showed that the toxingene of *S.aureus* isolates was found mainly in the wound and blood samples, suggesting that the toxin genes are very important and play essential role in the survival of *S. aureus* strains at these sites.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Rahimi F, Bouzari M, Katouli M, Pourshafe MR. Prophage and antibiotic resistance profiles of methicillin-resistant Staphylococcus aureus strains in Iran. AdvVirol. 2012;157(9): 1807-1811. 3.
2. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. ClinMicrobiol Rev. 2000; 13:16-34.
3. Baba-Moussa L, Anani L, Scheftel JM, Couturier M, Riegel P, Haeıkou N, Hounsou F, Monteil H, Sanni A, Pre´vost G, Virulence factors produced by strains of Staphylococcus aureus isolated from urinary tract infections. Journal of Hospital Infection. 2008; 68: 32-38.
4. Ferry T, Perpoint T, Vandenesch F, Etienne J. Virulence determinants in Staphylococcus aureus and their involvement in clinical syndromes. Curr Infect Dis Rep. 2005; 7: 420-428.
5. Puah S, Chua K, Tan J. Virulence factors and antibiotic susceptibility of Staphylococcus aureus isolates in ready-to-eat foods: detection of S. aureus contamination and a high prevalence of virulence genes. Int. J. Environ. Res. Public Health. 2016; 13(2):199.
6. Mehrotra M, et al. Multiplex PCR for Detection of Genes for Staphylococcus aureus Enterotoxins, Exfoliative Toxins, Toxic Shock Syndrome Toxin 1 and Methicillin Resistance. Journal of Clinical Microbiology. 2000; 38(3): 1032-1035.
7. Eshraghi S, et al. Prevalence of tst, entC, entA and entA/C genes in Staphylococcus aureus strains isolated from different foods". Tehran University of Medical Sciences. 2009; 67(7): 470-476.
8. Vasconcelos NG and de Souza MdLR. Staphylococcal enterotoxins: molecular aspects and detection method. Journal of Public Health and Epidemiology. 2010; 2(3) 29-42.
9. Karahan M, et al. Investigation of toxin genes by polymerase chain reaction in Staphylococcus aureus strains isolated from bovine mastitis in Turkey. Foodborne Pathogens and Disease. 2009; 6(8): 1029-1035.
10. Dinges MM, Orwin PM, Schlievert PM. Exotoxins of Staphylococcus aureus. Clin. Microbiol. Rev. 2000; 13: 16-34.
11. Cunha MLRS, Calsolari R.A. Toxigenicity in Staphylococcus aureus and coagulase-negative staphylococci: epidemiological and molecular aspects. Microbiol Insights. 2008; 1: 18-26.
12. Argudín MA, Mendoza MC, González-Hevia MA, Bances M, Guerra B, Rodicio MR. Genotypes, exotoxin gene content and antimicrobial resistance of Staphylococcus aureus strains recovered from foods and food handlers. Applied Environ Microbiol. 2012; 78: 2930-2935.
13. . Cheesbrough M. District laboratory practice in tropical countries. Cambridge: Cambridge University Press. 2006.
14. Kandala NJ, Abdulateef M, Imad N. Genotyping of Staphylococcus aureus Isolates Based on MethicillinResistance Genes and its Relatedness to some Putative Virulence Factors. Iraqi Journal of Science. 2017; 58(2A): 626-638
15. Sina H, Ahoyo TA, Moussaoui W et al. Variability of antibiotic susceptibility and toxin production of Staphylococcus aureus strains isolated from skin, soft tissue and bone related infections. BMC Microbiology. 2013; 13(1): 188.
16. Wang LX, Hu ZD, Hu YM, et al. Molecular analysis and frequency of Staphylococcus aureus virulence genes isolated from bloodstream infections in a teaching hospital in Tianjin, China. Genet Mol Res. 2013; 12(1): 646-654.
17. Plano LR. Staphylococcus aureus exfoliative toxins: how they cause disease. J. Invest. Dermatol. 2004; 122: 1070-1077.
18. Kacou N, Koffi K, Ekaza E, Kouamé E, Anne B, Dosso M. Staphylococcus aureus infection and virulence genes in Abidjan (Côte d'Ivoire). Eur J Sci Res 2011; 52: 339-344
19. Sabouni F, Mahmoudi S, Bahador A, Pourakbari

B, Sadeghi RH, Ashtiani MTH, Nikmanesh B, Mamishi S. Virulence Factors of Staphylococcus aureus Isolates in an Iranian Referral Children's Hospital. Osong Public Health Res Perspect. 2014; 5(2): 96-100.

20. Ikeda T, Tamate N, Yamaguchi K, Makino S: Mass Outbreak of Food Poisoning Disease Caused by Small Amounts of Staphylococcal Enterotoxins A and H. Appl Environ Microbiol. 2005; 71: 2793-2795.
21. Ali OA, Ogbolu DO, AO, Okorie AN, Aknola JO, Daniel JB. Asocition of virulence genes with mecA gene in Staphylococcus aureus isolates from Tertiary Hospitals in Nigeria. Indian J PatholMicrobiol. 2015; 58: 464-471