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Laboratory Based Diagnosis of Bacteraemia among Inpatients and Outpatients with Acute Febrile Illness at Khwaja Yunus Ali Medical College and Hospital in Bangladesh

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ABSTRACT

Bacteraemia is one of the important causes of mortality and morbidity worldwide. The emergence of multidrug-resistant bacterial strains is a major problem in its management. To encourage the prudent use of appropriate antibiotics in tertiary care study was undertaken. A total of 282 patients with suspected bacteraemia from indoor and outdoor patients were included in this study. Blood samples were processed in the microbiology laboratory and bacteria were recognized by standard laboratory methods and then antibiotic susceptibility test was performed following CLSI guidelines. Out of a total of 282 blood samples, 55(19.50%) were culture positive. Among 55 positive cultures, Gram-positive *Cocci* and Gram-negative *Bacilli* were 48(87.3%) and 7(12.7%) respectively. The most predominant organisms were *Staphylococcus aureus* comprising 42(76.36%), followed by *Salmonella typhi* 4(7.30%), *E. coli* 2(3.63%) and *Acinetobacter spp* 1(1.81%). Among the isolated *Staphylococcus spp*, only 6(10.9%) isolates were Coagulase-Negative (CoNS). In antibiotic susceptibility test, all of the isolated bacterial pathogens were found susceptible to imipenem and meropenem (100%). For *Salmonella typhi*, all 4 isolated strains were found resistant to amoxicillin, amoxicillin+clavulanic acid (amoxyclave) and cephradine and 3 out of 4(75%) were susceptible to ciprofloxacin and levofloxacin. Most of the Gram-positive *Cocci* (GPC) were susceptible to levofloxacin (90%), ciprofloxacin (85%), and amikacin (83%). Both of *E. coli* and *Acinetobacter spp* were sensitive to amikacin (100%). This study stresses the need for the continuous screening and surveillance for antibiotic resistance that would influence appropriate empiric treatment and infection control strategies for bacteremic cases.

Keywords: Bacteraemia, BD BACTEC™ FX40, Antimicrobial susceptibility patterns, and Diagnosis.

1. INTRODUCTION:

Bacteria in blood is bacteraemia, is a significant reason for morbidity and mortality in basically sick patients across the world (Vincent *et al.*, 2009). Human blood has generally been viewed as an altogether sterile condition; the recognition of organisms in blood has been reliably deciphered as a sign of infection (Doern, 2016). Bacteraemia happens

in illnesses, for example, typhoid fever, brucellosis, leptospirosis, and endocarditis (Cheeseborough, 2006). Blood culture techniques were used to find out the common bacterial isolates are *Staphylococcus aureus*, *E. coli*, Coagulase-Negative *Staphylococci* (CoNS), *Enterococcus spp*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Proteus spp*, and β -hemolytic *Streptococci*. CoNS have long been considered mainly as non-pathogenic blood

culture contaminant, however as a result of combination of increased use of intravascular devices and an expansion in the quantity of hospitalized immune compromised patients, CoNS has emerged as a significant reason for nosocomial blood stream infection (Assad, 2015).

In Bangladesh, data on the burden of bacterial infection in blood stream among children and adults attending outpatient clinics in rural hospitals is insufficient (Rabiul *et al.*, 2020). At present, for timely interventions of such cases bacterial contaminations, local predominance and antimicrobial activity has been analyzed (Abedin *et al.*, 2020). This investigation was led to distinguish the bacteriological profile in blood culture and anti-biogram designs out of the specimens collected from patients in a tertiary care hospital to guide clinicians to initiate empiric antibiotic therapy and to formulate antibiotic policy.

2. MATERIAL AND METHODS:

2.1 Study participants: The data was collected retrospectively from the register of the Hospital's Microbiology Laboratory that included both in-patients and out-patients with acute febrile illness between August and December 2019 at Khwaja Yunus Ali Medical College Hospital (KYAMC & H). The inclusion criteria were all the patients attending at KYAMC & H with a history of fever irrespective to their age. Blood samples (n=282) were aseptically collected from patients with suspected bloodstream bacterial infection out of all aged group.

2.2 Blood sampling and laboratory investigations: Aseptically blood samples were drawn for culture by automated system. A 1-5 ml and 8-10 ml amount of blood samples were inoculated into BD BACTEC™ FX40 Peds Plus/F for 0-12 year's children and BD BACTEC™ FX40 Aerobic/F culture vials for adults more than 12 years old. Only aerobic blood cultures were used in this study. Then specimens were quickly incubated at 35±2°C in the BD BACTEC™ FX40 Instrument (Becton, Dickinson and Company, Spark, USA) culture system for a maximum of 3 days unless flagged positive. Positive vials were sub-cultured on Blood agar, MacConkey agar, and Chocolate agar

following routine microbiological techniques prescribed by CLSI. Klignar Iron agar (KIA), MIU, and Simon citrate agar tubes, Oxidase tests were used for biochemical tests to identify the isolated pathogens. Specific antisera (Becton, Dickinson and Company, Spark, USA) were used for confirmation of *Salmonella spp.*

2.3 Antibiotic sensitivity test: *In-vitro* antimicrobial susceptibility testing for all the bacterial isolates was performed using the Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) as per CLSI guidelines (CSLI, 2015). The antibiotics used in the test included amoxicillin (10µg), cefoxitin (30µg), ceftriaxone (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), levofloxacin (5µg), cephadrine (30µg), meropenem (10µg) and imipenem (10µg). *Salmonella typhi* (ATCC 14028) and *Staphylococcus aureus* (ATCC 25923) were used as a control throughout the study for culture and antimicrobial susceptibility test.

2.4 Statistical Analysis of Experimental Data: Data obtained were analyzed by SPSS version 20 and Excel 2016. Descriptive statistics and chi-square tests were done to check the statistical evaluation. The p-value that considered significant was <0.5.

3. RESULTS:

In this study, a total of 282 clinical blood samples were analyzed by BD BACTEC™ FX40 from the hospitalized and outdoor patients. Among them, 55(19.50%) were blood culture positive cases. Out of 55 positive cultures, 31 (56.4%) were males while 24(43.6%) were females. The positive samples belonged maximum to adults 49(89.1%) as compared to children 6(10.9%). Out of 55 positive isolates, 48 (87.3%) were Gram-positive cocci, and 7(12.7%) were Gram-negative *Bacilli*.

The most predominant pathogens were *Staphylococcus aureus* 42(76.36%), followed by *Salmonella typhi* 4(7.30%) and *E.coli* 2(3.63%) and *Acinetobacter* species 01 (1.81%). Moreover, contaminants such as Coagulase-Negative *Staphylococci* (CoNS) were found in 6 (10.90%) isolates which were presented in **Table 1**.

Table 1: Frequency of bacterial isolates according to age groups.

Isolated pathogens	Age group				Total N (%)
	0-12 years	13-24 years	25-36 years	>37 years	
<i>Staphylococcus aureus</i>	6	5	10	21	42(76.36)
<i>Salmonella spp.</i>	0	0	2	2	4(7.30)
<i>Acinetobacter spp.</i>	0	0	0	1	1(1.81)
<i>E.coli</i>	0	0	1	1	2(3.63)
Contaminants:					
CoNS	0	1	0	5	6(10.90)
Total	6	6	13	30	55(100%)

Table 2: In-vitro Anti-biogram susceptibility patterns of Gram-negative and Gram-positive bacteria isolates.

Bacteria isolates	No/%	AK	AMX	AMC	CRO	CIP	CN	CXM	CFM	CE	IPM	MEM	LEV
<i>Salmonella typhi</i> (n=4)	No	2	0	0	1	3	1	2	0	0	4	4	3
	%	50	0	0	25	75	25	50	0	0	100	100	75
<i>Staphylococcus aureus</i> (n=42)	No	35	23	24	29	36	31	29	18	16	42	42	38
	%	83.33	54.8	57.1	69.0	85.7	73.8	69.0	42.9	38.10	100	100	90.50
<i>E. coli</i> (n=2)	No	2	0	0	0	1	0	0	0	0	2	2	1
	%	100	0	0	0	50	0	0	0	0	100	100	50
<i>Acinetobacter spp.</i> (n=1)	No	1	0	1	1	0	0	0	0	1	1	1	1
	%	100	0	100	100	0	0	0	0	100	100	100	100

No=Number, %=Percentage, AK=Amikacin, AMX=Amoxycillin, AMC=Amoxyclave, CRO=Ceftriaxone, CIP = Ciprofloxacin, CN=Gentamycin, CXM= Cefuroxime, CFM=Cefixime, CE=Cephadrine, IPM=Imipenem, MEM = Meropenem, LEV=Levofloxacin

Antibiotic susceptibility test illustrated that the most sensitive antibiotics against *Salmonella typhi* and *Staphylococcus aureus* were imipenem and meropenem with a susceptibility rate of 100% in both cases followed by levofloxacin 3 (75%) for *Salmonella spp* and 38 (90.5%) for later pathogen. On the other hand, antibiotics such as amoxicillin, amoxyclave, and cepharadine were found to be least effective with 100% resistance against the *Salmonella species* pathogen. All Gram-negative bacteria isolates were found in blood culture were observed to have high rates of resistance to amoxicillin (Table 2).

4. DISCUSSION:

Blood stream infections (BSI) can range from the in apparent bacteraemia to fulminates septic shock. Blood culture has been the most effective test with high affectability and explicitness to identify the etiologic agents in blood stream infection among all other relevant tests. The detection system is critical for making decision of administering proper antimicrobial treatment. Besides, quick and early detection of bacterial isolates could save the fundamental organs of body.

In our analysis, blood culture positivity rate was 19.50 % which is consistent with results of other studies led by Vijaya Devi *et al.* (2015), Qureshi *et al.* (2011) and Mehta *et al.* (2005), and that were 16.8%, 16.6% and 16.4% separately. The limited number of positive cases of blood culture might be clarified by the way that a significant number of the patients likely got antimicrobial medications before they sought treatment to tertiary health care facilities (Md *et al.*, 2014).

In the study, among all positive cases 56.4% were males and 43.6% were females having a slight predominance of males than females with a ratio of 1.3:1. This outcome was predictable with the investigation done by Vanitha Rani *et al.* (2012) where 60.2% was male and 36.7% was female. A comparative report was finished by Kaur and Singh (2014) who revealed high culture positive rate at 65.22% in men. Also, the study reported the presence of medically important Gram-positive and Gram-negative bacteria in blood stream with 87.27% and 12.73 % respectively; comparable to isolation rates accounted by Durmaz, *et al.* (2003).

However, in some investigations as announced by Mehta M *et al.* (2005), Gram-negative microorganisms were found as high as (80.96%) as compared to 18% of Gram-positive microbes. *Staphylococcus aureus* was the most predominant Gram-positive bacteria (76.36%) found in our analysis. This is overwhelming in all age groups being most elevated in adults. This perception is in non-concordance with different examinations led by Oza Sweta *et al.* (2016). *Salmonella typhi* were the second common isolated pathogens (7.30%) found in adults in our study. In our examination, 10.9% of Coagulase Negative *Staphylococci* (CoNS) were found as near about concurrence with considers led by Gandham Pavani *et al.*, (2012) (23%) Sharma *et al.* (13.3%) (2015), S. Oza Sweta *et al.* (20.2%) (2016).

However, some investigators had revealed low event of CoNS, 7.1% and 5.6% individually by Meenakshi Kante *et al.* (2014). The most skin commensal is CoNS may be contamination of blood culture vial due to not following aseptic technique during blood collection.

The true blood pathogens of CoNS are increased because of using intravascular devices.

In this study, we found that all of the bacterial isolates were fully sensitive (100%) to imipenem and meropenem. *Salmonella typhi* showed highest resistance to amoxicillin, amoxyclove, and cephadrine (100%) followed by ciprofloxacin and levofloxacin (75%). The resistance patterns of amoxicillin were observed in all isolated bacterial pathogens in this work.

In our investigation, *Staphylococcus aureus* was the largest isolated bacterial pathogen and the most sensitive to levofloxacin (90.5%) followed by ciprofloxacin (85.7%), amikacin (83.3%), and gentamycin (73.8%). The least sensitive antibiotics against the bacteria were cefixime (42.8%) and cephadrine (38.1%). These significant levels of resistance from regularly utilized anti-microbials were also observed in other similar studies conducted by Vanitha Rani *et al.* (2012).

The study also reported highest susceptibility of amikacin, imipenem and meropenem (100%) against other Gram-negative isolates like *E. coli* and *Acinetobacter* species which is predictable with the examinations directed by Mustafa *et al.* (2014), and Sanjay D Rathod *et al.* (2012).

5. CONCLUSION:

In conclusion, we have performed blood culture by automated BD BACTEC FX™ 40 and *in vitro* analysis of antibiogram patterns from patients with acute febrile illness sought treatment at tertiary level hospital in Bangladesh. The blood culture analysis revealed 19.5% (55/282) positive bacteraemia patients with a number of suspected isolates were *Staphylococcus aureus*, *Salmonella typhi*, *E. coli* and *Acinetobacter spp.* The fourth generation antibiotic Carbapenem such as imipenem and meropenem were highly sensitive (100%) to against all of the bacterial pathogens and amoxicillin and amoxyclove were found the least sensitive antibiotics. The presence of antibiotic resistant isolates imposes a serious concern about the drug of choice for treatment of bacteraemia in patients.

A careful consideration ought to be taken before deciding the empirical antibiotic for treatment in order to prevent the emergence of anti-microbial resistance and significant mortality and morbidity associated with the illness.

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7. CONFLICTS OF INTEREST:

The authors declare that they have no conflicts of interest.

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