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Comparison of automated and conventional blood cultures and their antibiotic resistance patterns in *Salmonella enterica* Typhi serovar isolates in a tertiary care centre.

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Abstract

Background and Objective:Enteric fever, often known as typhoid and paratyphoid fever, is a potentially fatal disease caused by Salmonella serotypes Typhi and Paratyphi, respectively. In developing and impoverished nations, it is a serious public health concern.This study aims to evaluate antibiotic resistance patterns and compare the automated blood culture system against the traditional blood culture method.Material method: The current study was conducted in the Mahatma Gandhi Memorial Medical College's microbiology department in Indore, Madhya Pradesh, central India, between 2020 and 2021. Blood samples from suspected enteric fever cases were received by the microbiology department. Twenty-six isolates of salmonella species were identified from 140 blood samples; these isolates were included in the study. Antimicrobial susceptibility testing was performed for all isolated organisms on Mueller-Hinton agar by the Kirby-Bauer disc diffusion method as per CLSI M100 2020 guidelines.Result: This study involved the collection of blood in BHI broth for the purpose of culture, as well as the testing of antibiotic sensitivity in 140 clinically suspected cases of enteric fever that were received by the Department of Microbiology at M.G.M. Medical College, Indore. 26 Salmonella enterica strains that were culture-positive were isolated and identified out of the 140 blood culture samples that were received.Conclusion: Salmonellosis is still a concern for public health. It is imperative to fortify preventative measures, enhance low-income nations' access to diagnostic resources, and encourage careful administration of antibiotics.

Key words:Blood culture, antibiotic resistance, BHI

Introduction

Enteric fever is a systemic bacterial infection caused by both *Salmonella enterica* serovar typhi and other serovar paratyphi A, B, and C. [1]. The developing world has 100–1,000 cases of typhoid fever for every 100,000 people. [2]. The illness is more common in developing nations with poor sanitation and limited access to safe drinking water. The disease is primarily linked to travel to endemic areas and has a lower incidence in developed nations. [3].Microorganisms found in a patient's blood have significant implications for diagnosis and prognosis. Blood cultures aid in the identification of organisms and support the treating physician in the timely and effective administration of antibiotics. [4]. Blood culture is considered the gold standard for identifying the causative factors of bloodstream infections. It is fast, affordable, and precise, with a sensitivity of 35–90%. There are several methods used to perform blood cultures, from fully automated to conventional[5]. The conventional method for blood culture is routinely followed in laboratories, where the blood sample is added to 100 ml of Brain Heart Infusion broth and incubated at 37°C for 24 hours. The bottles are observed regularly for signs of growth, and when there is evidence of growth, the laboratory does subculture on solid media [6]. Recognising the limitations of the conventional method, there is a demand for a more advanced diagnostic tool offering increased yield and speed. The automated blood culture system emerges as a promising innovation in the diagnosis of bloodstream infections, providing continuous monitoring with heightened sensitivity, specificity, and a faster turnaround time. [7, 8, 9, 10]As the burden of antimicrobial resistance in Salmonella is rising, there is a surge in multidrug resistance (MDR) and fluoroquinolone resistance infections, which lead to adverse clinical outcomes [11, 12]. *Salmonella's* species distribution and pattern of antibiotic resistance change with location and time. Research conducted in our nation has revealed that the MDR range for *Salmonella* is between two and four percent. [13, 14].An enhanced understanding of the antibiogram and spectrum of the infectious agent specific to a given area would facilitate patient diagnosis and treatment, as well as the development of hospital antibiotic policies. In order to identify microbial pathogens in bloodstream infections, the purpose of this study is to compare the automated blood culture system with the conventional blood culture system.

Material method

The present study was carried out from 2020 to 2021 in the department of microbiology at Mahatma Gandhi Memorial Medical College, Indore, Madhya Pradesh, central India. Blood samples were received from suspected cases of enteric fever in the department of microbiology. A total of 140 blood samples

were taken, out of which 26 isolates found to be *salmonella* species were enrolled in this study.

Inclusion criteria Clinical specimens of suspected cases of enteric fever received in the department.

Exclusion criteria1. Non-typhoidal isolates. 2. Salmonella isolates from environmental samples like food, water, etc.

Procedure Withdraw 2 mL–5 mL of venous blood from children and 05 mL–10 mL of blood from adult patients suspected of enteric fever. Brain heart infusion (BHI) broth bottles (Bijou bottles) or automated blood culture bottles (BacTalert) containing blood samples were received in the laboratory. Incubate the BHI bottle at 37 ^{O}C for 18–24 hours and follow for 7 days. Perform the first blind subculture on solid agar media, namely blood agar and MacConkey agar, after 18–24 hours of incubation, and then on every alternate day until day 7. Examine the BHI broth bottle daily for any visible signs of growth like haemolysis, turbidity, gas formation, pellicle formation, clotting, etc. For automated blood culture bottles, look for flagging at least daily, and once it flags positive, do gram staining, culture, and identification. Automated blood culture bottles also need to be followed for 7 days. Identification of the organism was done by colony morphology, Gram staining of the marked isolated colony, motility testing (hanging drop), and standard biochemical tests.
Antimicrobial susceptibility testing was performed for all isolated organisms on Mueller-Hinton agar by the Kirby-Bauer disc diffusion methodas per CLSI M100 2020 guidelines. The antibiotic discs with appropriate content were used and interrupted according to clinical and laboratory standards institute guidelines [15].

Statistical analysis: The data were analysed using SSPS version 22. Frequencies and percentages were used to describe the categorical variables in this study. The results were presented as proportion ratios with a 95% confidence interval. P098Statistical significance was set if p-value <0.05.

Result

In this study, blood in BHI broth was collected for culture and antibioticsensitivity was performed and analyzed from 140 clinically suspectedentericfevercases,whichwerereceivedintheDepartmentofMicrobiology, M.G.M.Medical College,Indore.From 140 blood culture samples received, 26 culture positive *Salmonellaenterica* strains were isolated and identified. The rate of isolation was18.6%.(n=26).Hundred percent (n=26) of the isolates of *Salmonella enterica* species, were identified as serovar Typhi. *Salmonella* Paratyphi was not isolated in this study.

Table 1. PercentageofBloodCulturePositives

	No.ofCases	**Percent**
*S.*Typhi	26	18.6
Sterile	114	81.4
Total	140	100.0

Table 2. Comparison of blood culture positivity by automated andconventional techniques.

	Positive	**Negative**	**Total**
Automatedbloodcul ture	16	34	50
Conventionalbloodcultu re	10	80	90
Total	26	114	140

Chi-squarestatisticsis9.27.**Thep-valueis0.002**.Theresultissignificant

Table 3. Antimicrobialsusceptibilitypatternin*Salmonella*Typhi

AntibioticDisc	***S.*Typhi(n=26)**		
	Sensitive	**Intermediate**	**Resistant**
Chloramphenicol(30 µg)	26(100%)	-	0(0.0%)
Ceftriaxone(30µg)	25(96.1%)	1(3.84%)	
Ampicillin(10µg)	26(100%)	-	0(0.0%)
Cotrimoxazole(1.25/23.75µg)	26(100%)	-	0(0.0%)
Azithromycin(15µg)	24(92.30%)	-	2(7.69%)
Ciprofloxacin(5 µg)	09(34.61%)	-	17(65.38%)

Discussion

Enteric fever is a global health problem. It more commonly affects peopleindevelopingcountrieslikeIndia.Drugresistanceby*Salmonellaentericas*peci

estoantibioticshasledtotreatmentfailureandthuscausing mortalityandmorbidity.Inthisstudybloodcultureswereprocessedbothin BHIbroth bottleandin automatedblood culture bottles. The isolation of*Salmonella*Typhiwasbetterbyautomatedbloodculturetechnique(31%)thanbyc onventionalbloodculturemethod(11.11%).When comparing the automated culture approach with the conventional method, a significant correlation was found (p-value = 0.002). which is comparable to the research done by Karen K. Krisher et al., wherein 29% of pathogens are isolated by automated systems and just 10% are isolated by conventional systems[16].Similarly, a study done by Appiah GD *etal*Shows 28% positivity. And Ahirwar SK *etal*Showed 8.8% positivity by conventional method, Lekshmi L. Rajan (2017) *etal*showed 27.8% isolation by automated blood culture method [17, 18, 19].There are various advantages of automated blood culture over the conventional method, like continuous monitoring of the bottle by the machine, and it flags as soon as the organism is detected. Thus, the time required for reporting is much less than with the conventional method. The automated method is more sensitive and gives a higher culture of positivity as compared to the conventional method. It saves a lot of manpower as it is less labour-intensive. Also, the chances of contamination are higher with conventional methods than with automated systems.The 26 isolated strains of *S*. Typhi in this study were 96.1% sensitive to ceftriaxone, 3.84% were intermediate sensitive to ceftriaxone, 92.30% were sensitive, and 7.69% were resistant to azithromycin.In comparison with Sunil Poudel *et al.* (2014), they showed 93% sensitivity to azithromycin [20].In the current study, no isolates showed resistance to ceftriaxone; similarly, multiple studies showed 100% sensitivity to ceftriaxone [21, 22].The high resistance to any antibiotic can be attributed to rampant, irrational, and overuse of them. There is an emerging resistance to ceftriaxone in *S. Typhi, which* is attributed to ESBL. AmpC beta-lactamase production in *S.* typhi is also responsible for resistance to broad-spectrum cephalosporin.One isolate, which, while performing AST by the Kirby-Bauer method in our study, was found to have a zone diameter falling in the intermediate zone for ceftriaxone, was sent to CMC, Vellore, for confirmation and gene detection. They performed the Kirby-Bauer method and MIC and found them to be sensitive. ESBL and Amp C gene PCR were also found to be negative at CMC, Vellore. In a study by Fernanda Marques Fitch in 2016, *Salmonella*, which were resistant and intermediate, were subjected to the detection of resistance genes, and the resistance genes were detected in 45 isolates. [23]. In this study, 65.38% of ciprofloxacin resistance was found. Comparative results were found in studies by Balaji Veeraraghavan *et al.* (2016) and Ruchi Girotra *et al.* (2016), which showed a higher percentage of resistance to Ciprofloxacin, which was around 65% [22, 24].The majority were resistant to ciprofloxacin, which indicates misuse of these drugs to treat febrile illnesses of assumed bacterial origin. *Salmonella*

spp. continues to be an important cause of BSI and poses a major health problem. Antimicrobial resistance is becoming more common, which is a global concern with potentially disastrous consequences for impoverished nations. Therefore, to detect, characterise, and capture the actual burden and manage the BSI and antibiotic resistance of *Salmonella*, upgrades to the diagnostic and surveillance systems are required.

Conclusion

Salmonellosis remains a public health problem. An automated blood culture system isolates organisms more quickly and precisely than a typical blood culture method.The susceptibility patterns of isolated enteric fever pathogens must be continuously monitored due to their varying degrees of antibiotic resistance. To stop the establishment of antibiotic resistance and future outbreaks, cautious use of antimicrobials, dedicated infection control procedures, and strict antibiotic policy should be put into place.

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