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(RESEARCH ARTICLE)

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Antimicrobial susceptibility pattern of bacteria isolated from clinical samples

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Abstract

The development of resistance to multiple drugs is a major problem in the treatment of infections by pathogenic microorganisms. Multidrug efflux pump helps the bacteria for antibiotic resistance. Bacterial cell have intrinsic capacity to restrict the entry of small molecules. These property is more pronounced in Gram negative bacteria, whose outer membrane provides an effective barrier and constitutes first line defense against antimicrobial challenge. Gram positive bacteria lacks the outer membrane and hence lacks first line defense. Rapid detection and identification of clinically relevant microorganisms in blood cultures is very essential and determination of antimicrobial susceptibility pattern for rapid administration of antimicrobial therapy has been shown to reduce the morbidity and mortality associated with bloodstream infections. So, this study was undertaken to investigate the type of bacteria isolated from cases of bloodstream infections and determination of their antibiotic susceptibility pattern. Out of 15 isolates, 9 were found to be resistant to the antibiotic tested.

Keywords: Antimicrobial; Drug Resistance; Antibiogram; Bacteria

1. Introduction

Bacteria play a very vital role in recycling nutrients. While majority of bacteria in the human body are countered by the immune system, there are a few that are pathogenic in nature. Pathogenic bacteria cause infectious diseases like leprosy, cholera, anthrax and bubonic plague. They are also responsible for the spread of respiratory infections like tuberculosis. Use of antibiotic to protect from such diseases caused by bacteria was carried out after 2nd world war. The first antibiotic was penicillin, discovered accidentally from a mold culture. The publication on penicillin by Alexander Fleming in 1928 is a milestone in the history of medicine. As more antimicrobial compounds were discovered, it was predicted that infectious diseases would be eliminated through the use of these antimicrobials. Unfortunately, the development of bacterial resistance to these antimicrobials quickly diminished this optimism and resulted in the need for physicians to request the microbiology lab to test a patient's pathogen against various concentrations of a given antimicrobial to determine susceptibility or resistance to that drug. Today various different antibiotics are available to doctors to cure minor discomforts as well as life-threatening infections [1]. Widespread antibiotic resistance is one of the hallmarks of the third epidemiological transition, where populations move from a chronic disease burden back to an increasingly uncontrollable burden of infectious disease perception, however, is that the problem is seen as exclusively associated with the use and overuse/ misuse of antibiotics in humans and animals. As the degree of antibiotic resistance rate for blood stream pathogens is alarming, it is mandatory to monitor the susceptibility of these isolates in order to avoid inappropriate use of antibiotics in hospital wards [2] .

Blood stream infections (BSI) are important cause of morbidity and mortality worldwide, for example in the USA, it is estimated that about 10 to 20% of nosocomial infections are due to BSI [2]. Rapid detection and identification of clinically relevant microorganisms in blood cultures is very essential and determination of antimicrobial susceptibility

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pattern for rapid administration of antimicrobial therapy has been shown to reduce the morbidity and mortality associated with bloodstream infections [3] So, this study was undertaken to investigate the type of bacteria isolated from cases of bloodstream infections and determination of their antibiotic susceptibility pattern.

2. Materials and Methods

2.1. Collection of samples

The blood samples were collected from central laboratory and urine, sputum and pus sample was collected from microbiology laboratory of Badnapur city.

2.2. Enrichment and isolation of clinical strains.

These samples were plated on presterilized nutrient agar, Luria bertani agar and blood agar and incubated for 37°C to 48 hrs. After incubation, 8 blood, 3 urine, 2 sputum, and 2 pus isolates with biochemical identification and morphological appearance were selected and maintained on nutrient agar slants at 4°C until further use.

2.3. Antibiotic susceptibility testing and antibiogram profiling

Antimicrobial susceptibilities of the isolates were determined by disc diffusion methods on Nutrient agar (HiMedia, India) by using commercial antibiotic discs (HiMedia, India). The antibiotic discs used for the isolates were penicillin (P) 30 µg/ml, ampicillin (Am) 10 µg/ml, Streptomycin (St) 25 µg/ml, Tetracyclin respectively. Each plate spreaded with the respective isolates and incubated for 24 h aerobically at 37°C. Susceptibility testing of each drug for each isolate was performed three times under the same conditions. Zone diameter of inhibition of each isolate to the disc was read with a calibrated ruler. [4]

3. Result

3.1. Morphological characterization

Total 15 microbial isolates were obtained clinical samples i.e Blood (n= 8), urine (n=3), sputum (n=2), pus (n=2). Isolates were subjected to gram staining, Isolates from blood were gram positive (n=3), gram negative (n=5);From urine were gram positive (n=2), gram negative (n=1);From sputum were gram positive (n=2), gram negative (n=0);From pus were gram positive (n=1), gram negative (n=1). The results were shown in table 1.

Table 1 Gram nature and morphological characterization of the isolates

Samples	Grams nature	Morphology
B1	-ve	Cocci in cluster
B2	-ve	Rods in chain
B3	-ve	Diplococcus
B4	+ve	Cocci in cluster
B5	+ve	Short rod
B6	+ve	Cocii
B7	-ve	Cocii in cluster
B8	-ve	Cocci in cluster
U1	-ve	Short rod
U2	+ve	Cocci in chain
U3	+ve	Cocci in cluster
S1	+ve	Large cocci
S2	+ve	Short rods

P1	-ve	Cocci in cluster
P2	+ve	Cocci in cluster

3.2. Antibiotic susceptibility testing

Antibiotic susceptibility testing was done with the help of standard antibiotics. Out of 15 isolates B3, B4, B5, B7, B8, U1, U2, S1, P1 were found to be resistant to particular antibiotics. The result of antibiotic sensitivity testing were given in table 2

Table 2 Antibiotic susceptibility testing of the isolates

	Streptomycin	**Penicillin**	**Tetracycline**	**Amphicillin**
B1	2.13	3.93	2.26	3.06
B2	1.46	1.46	1.8	1.66
B3	2.66	2.53	1.6	2.06
B4	2.46	R	1.66	0.66
B5	1.4	R	0.93	R
B6	2.46	2.93	1.86	2.2
B7	1.86	R	1.93	3.8
B8	1.8	R	3.06	R
U1	1.53	R	R	1.53
U2	R	R	R	R
U3	2.33	0.4	R	0.86
S1	1.93	R	0.26	R
S2	1.86	0.8	0.66	0.66
P1	1.16	R	0.66	R
P2	1.9	0.6	1	0.33

4. Discussion

In this study agar disc diffusion method was used for the susceptibility testing. Various methods are available for the susceptibility testing. Disc diffusion methods are suitable for organisms that grow rapidly at 35-370C. The techniques are technically simple, cheap and reliable, but there is no single internationally accepted method of disc diffusion testing. In the United Kingdom majority of laboratories use the modification of Stoke's disc diffusion method [4] (originally started to use for primary cultures where inoculum standardization could not be ensured) and BSAC (British Society of Antimicrobial Chemotherapy) methods. However, agar disc diffusion is accepted as a standard operating procedure (SOP). Many countries including France, Germany, Sweden, and US have adopted disc diffusion method of CLSI (Clinical and Laboratory Standards Institute) and the updates are published every 2-3 years which are accepted world-wide including the laboratories in India.

Penicillin resistance among strains from nosocomial infections has remained high since it emerged about two decades ago and still remained high as reflected from the result of this study. Resistance to β-lactamase stable penicillins such as ampicillin has also increased. For example, resistance of clinical isolates to ampicillin which was 0% in 2009 [5] in this study isolates were found to be more resistance to amphicillin followed by penicillin. Which indicates that Beta-lactam resistance gene is highly present in all type of clinical samples. This might be due to variations in the usage of β-lactam and synthetic antibiotics in different geographical areas. This increase is coinciding with the paradigm shift from the use of β -lactam penicillins to the β-lactamase resistant penicillins for empirical treatment because of the ineffectiveness of β-lactam penicillins due to their destruction by β-lactamases [6]. The bacteria which were sensitive to tetracycline in the 1980s all over the world, especially in India [4], have gradually become resistant. The high

resistance rates to these antibiotics in our study are a reflection of the abuse and misuse as a result of poor antibiotic prescription policy and off-the-counter availability of these agents. Resistance of both Gram positive and Gram negative bacteria to newer broad spectrum antibiotics is also gradually developing.

One urine isolate was highly resistance against all used antibiotics which shows multidrug resistance pattern. Multiple drug-resistances detected in this study are an extremely serious public health problem that has been found associated with outbreaks of major epidemics worldwide. [7] The results of the present and earlier studies indicate that extensive use of chemotherapeutants has increased the incidence of drug resistance in clinical and environmental microbes. Infections with multiple drug-resistant bacteria have been recognized, and the increased prevalence of such strains has led to serious problems in standard antibiotic treatment. [8] .The excess antibiotics may remain in the aquatic environment for long period of time, and antimicrobial resistance may develop among bacteria in the exposed ecosystems. This resistance can be transmitted to a wide range of bacterial species including bacteria pathogenic to human [9].

5. Conclusion

The study highlights the alarming prevalence of antimicrobial resistance among bacterial isolates from clinical samples, underscoring the urgent need for continuous surveillance and rational antibiotic use. Out of the total 15 isolated bacteria, 9 bacterial species showed high resistance to commonly used antibiotics. The observed resistance patterns emphasize the importance of culture and sensitivity testing prior to initiating empirical therapy. Implementation of robust antimicrobial stewardship programs, along with strict infection control measures, is essential to curb the spread of resistant strains and to preserve the efficacy of existing antibiotics.

Compliance with ethical standards

Statement of informed consent

During the study an Informed consent was obtained from all individual participants, which gave blood to conduct the study.

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