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Significance of Modified Hodge Test in Carbapenemase Detection: A Brief Insight

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ABSTRACT

Neonatal sepsis is one of the leading causes of neonatal deaths. A. *baumannii-calcoaceticus* is the most notorious bacterial agent. Carbapenems are the most important antibiotics and modified Hodge Test is considered as important phenotypic method for observing Carbapenemase production. **Objective:** To evaluate the efficacy rate of Modified Hodge Test, for detection of Carbapenem resistance. **Methods:** A cross sectional study was conducted at department of pathology, Nishtar Medical University, Multan from August 2023 to September 2023. The blood samples of suspected cases of sepsis were collected and after isolation of *Acinetobacter baumannii* sensitivity of multiple antibiotics were checked by disc diffusion method. Carbapenem resistance was re-evaluated by Modified Hodge Test using Meropenem disc (10 µg). All data were entered and analyzed by SPSS version 23.0. **Results:** Total samples of neonatal sepsis were 182. 83 (45.6%) were culture positive for bacterial growth. Among the positive samples 26 (31.3%) were isolated as *Acinetobacter baumannii*. Kirby-Bauer disc diffusion method was used to check sensitivity of multiple antibiotics including Carbapenems. Out of 26 *Acinetobacter* isolated samples, 16 were found to be Carbapenem resistant by this method. Modified Hodge test was used to re-confirm Carbapenem resistance. Out of 16 Meropenem resistant cases this phenotypic test only detected 5 cases (31.25%). **Conclusions:** *Staphylococcus aureus* followed by *Acinetobacter baumannii* were isolated predominantly and Carbapenem resistance has markedly increased. In contrast to a study conducted in 2010 in Pakistan on MHT effectiveness where effectiveness of MHT for Carbapenemase detection was satisfactory, our results reveled that some other techniques should be introduced for Carbapenemase detection as Modified Hodge test did not give satisfactory results.

INTRODUCTION

Among various microorganisms involved in the several hospital-acquired infections *A. baumannii-calcoaceticus* is the most notorious bacterial agent. Major targets of this pathogen are those who are ill or have compromised immune system [1]. In tropical countries, long period existence of *Acinetobacter* pathogen at the multiple surfaces causes various outbreaks and infections mostly in ICUs [2]. There are multiple species of *Acinetobacter* but major pathogenic species is *Acinetobacter baumannii.* Hence, *A. baumannii* is frequently involved in most of the infectious diseases outbreaks [3]. Generally, these outbreaks are related to hospitals and water sources which create a challenging environment to put down pathogens as well as lessen the contamination in environment [4]. A. baumannii is a non-fermentative, and is the cause of several infections such as VAP, bacteremia, wound infection, UTI and several other nosocomial infections. The main concern of *A. baumannii* is its strong antimicrobial resistance. In ICU patients including those who undergo invasive procedures or encounter excessive broad-spectrum antibiotics, this Carbapenem Resistant *Acinetobacter baumannii* (CRAB) is associated with poor outcomes and high mortality (50%) as compared to those with bacteremia by other bacteria [5]. Neonatal sepsis is one of the leading causes of neonatal death [6]. In 2019, a study was conducted in which it was reported that the

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overall incidence of neonatal sepsis in Asian countries such as China, Thailand, Macau, and Malaysia was 26 per 1000 admissions [7, 8]. Hospital settings contain the flora which is highly resistant as many drugs are being introduced to patients and resistance is grossly increasing now a day. A. baumannii is among the bacteria which exhibited deadly resistance against multiple microorganisms. Various hospital acquired strains of *Acinetobacter* exhibit mechanisms of resistance including expression of β-lactamases, efflux pumping of antibiotic drug and alterations in cell-wall channels (porins) [9, 10]. Carbapenem and Cephalosporin resistances are associated to broad range of genetic matters. Among the transmissible oxacillinase, OXA-23-like was the most common. Additionally, the presence of ESBL was also found along in some isolates along with Carbapenemase [11]. Antibacterial drug resistance is an emerging issue. Currently, the first line therapies for suspected sepsis cases are engaging Piperacillin/Tazobactam and Amikacin [12]. However, when the empirical therapies consisting of first line drugs become unrewarding then Carbapenems are applied. In the most recent era, high Carbapenem resistance has left the clinicians with limited choices of antibiotics [13]. Carbapenems are the most important antibiotics as they can be used to treat severe infections. However, because of severe infection rates a serious public health threat has generated by the emergence of Carbapenem resistance [14]. This resistance mainly develops by the production of hydrolyzing enzymes (Carbapenemase). Uprising mortality rates due to Carbapenemase producing Carbapenem-resistant Enterobacteriaceae (CP-CRE) infection presents a great threat to population as compared to infections by non-CP-CRE. Moreover, high potential of Carbapenemase genes to get transferred by plasmids is an important factor [15]. CP-CRE is also less likely to be susceptible to other groups of antibiotics, including fluoroquinolone, polymyxins and aminoglycosides. Therefore, rapid and reliable detection of CP-CRE is great interest to streamline not only antibiotic treatment but also to minimize the spread of these bacteria both in health care facilities and in community [16]. Multiple methods are available for the detection CP-CRE, however Disk Diffusion Test and Modified Hodge Test are considered as important and accurate methods for the monitoring of Carbapenemase production. Thus, early and accurate identification of CP-CRE is extremely important not only for the treatment but for the control of spread and prevention of infections as well.

This study focused on the efficacy rate of Modified Hodge Test, for detection of carbapenem resistance.

METHODS

A cross sectional study was conducted at department of pathology, Nishtar Medical University, Multan from August 2023 to September 2023 and was approved by ethical review committee with a Ref. No. 13119/NMU. The sample size of the study was calculated by using online calculator openepi.com with 95% confidence level and p = 13.7% as expected proportion/anticipated frequency in population based study conducted by Nazir et al in 2019 [17]. Consent forms were available in the Neonatal ICU and parents/guardians were informed thoroughly about the study. After the approval of parents/guardians, blood samples of neonates were sent to laboratory with the filled history taking Performa and consent forms. According to the microbiological guidelines the blood samples of suspected cases of sepsis from neonatal ICU wards were collected by using aseptic techniques. Instantly, these samples were then transported to Microbiology lab for culture and sensitivity testing. As per microbiological protocols the samples were processed in VersaTREK Automated Microbial Detection System. The positive samples were next sub-cultured by streaking method on Blood agar and MacConkey agar. Cultured samples were further processed for biochemical testing. Genus Acinetobacter was distinguished on basis of gram staining and multiple biochemical tests. API 20E kits were used for reconfirmation of the species. Sensitivity of multiple antibiotics was checked by Disc Diffusion method. Antibiotic susceptibility testing was done following CLSI 2022 guidelines by using Kirby-Bauer disc diffusion method on Muller Hinton agar. Carbapenem resistance was also re-confirmed by Modified Hodge Test using Meropenem disc (10 µg). For that, 5ml saline was used to prepare 0.5 McFarland dilution of E. coli ATCC 25922. 0.5 ml of the 0.5 McFarland solutions was added dropwise to 4.5 ml of saline to make dilution of 1:10. A lawn of the dilution was streaked on Muller Hinton agar plate and allowed to dry for 3-5 minutes. 10ug Meropenem disc was placed in the middle of plate. Test organism was streaked from edge of disc to edge of plate. ATCC strain was also processed in same manner. The plate was incubated overnight at 37°C. Clover leaf pattern was observed at the corner junction of growth and streaked line. Efficacy of Modified Hodge test was interpreted in form of percentage for detection of Carbapenem resistance or Carbapenemase production. All data were entered and analysed by SPSS version 23.0.

RESULTS

Total samples of neonatal sepsis were 182. The mean age of neonates was 14.4 ± 6.39 days and majority 112 (61.5%) were male. Our study results showed that out of 182, 83 (45.6%) were culture positive for bacterial growth and 99 (54.4%) were culture negative. Among the positive samples 26 (31.3%) were isolated as *Acinetobacter baumannii*. Other isolated organisms include *Staphylococcus aureus* 29 (34.93%), *Enterobacter spp* 17 (20.48%), *Pseudomonas aeruginosa* 4 (4.81%), *E. coli* 3 (3.61%), *Klebsiella*

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pneumoniae 3 (3.61%) and *Citrobacter spp* 1 (1.2%). The specific isolated strains of *Acinetobacter* were evaluated for multiple variables. According to the data most affected gender was of males in neonatal sepsis. Gestational age showed that almost equal number of neonates fall in preterm (54.1%) and term (45.6%) categories. However, weight at birth had great impact on developing Acinetobacter associated neonatal sepsis as 86.8% were low birth weight (<2.5 kg) (Table 1).

Table 1: Demographic Details of Study Cases

S. No.	Variables	Frequency from Total Samples N (%)
Gender		
1	Male	112 (61.5)
2	Female	70 (38.5)
Gestational Age		
3	Preterm <37 Weeks	99 (54.1)
4	Term >37 Weeks	83 (45.6)
Age		
5	<7 Days (EOS)	99 (54.1)
6	7-29 Days (LOS)	83 (45.6)
Weight at Birth		
7	<2.5 Kg (Low)	158 (86.8)
8	>2.5 Kg (Normal)	24 (13.2)
Mode of Delivery		
9	C-Section	167 (91.8)
10	SVD (Normal)	15 (8.2)

Kirby Bauer disc diffusion method was used to check sensitivity of multiple antibiotics including Carbapenems. Out of 26 Acinetobacter isolated samples, 16 (61.53%) were found to be Carbapenem resistant. Modified Hodge test was used to re-confirm Carbapenem resistance. Out of 16 Meropenem resistant cases (confirmed by Disc Diffusion Method), this test only detected 5 cases (31.25%) (Table 2).

Table 2: Comparison of Disc Diffusion Method and Modified Hodge Test for Carbapenemase Detection

Number of Cases of *Acinetobacter baumannii*	Total Resistant *Acinetobacter* Cases N (%)	Disc Diffusion Method N (%)	Modified Hodge Test N (%)
	16 (100)	16 (100)	5 (31.25)

DISCUSSION

In our study Meropenem was resistant in 61.53% cases. In contrast to our study Gladstone *et al.*, from Vellore, India conducted a study in which they published 14% Carbapenem resistant Acinetobacter spp isolated from tracheal secretions samples [18, 19]. After that, in 2006 in New Delhi the prevalence of Carbapenem resistant Acinetobacter was found to be 35% [20]. Above studies showed upward trend in Carbapenem resistance pattern for Acinetobacter. In 1999, a surveillance study was conducted on Acinetobacter in which Amikacin 10%–51%, Piperacillin-Tazobactam 36%-75% and Imipenem 5%-19% were resistant [21]. In contrast to these results our study provided the data that Piperacillin-Tazobactam has less resistance i.e. 2% and Meropenem has become more resistant i.e. 61.53%. Amikacin was found to be at same resistance level of 30.76% which is in the range of former surveillance study i.e., 10%-51%. This high level resistance against multiple antibiotics has made Acinetobacter a popular and persistent hospital pathogen. The main cause of increased resistance to Carbapenems is excessive and unjustified use of Meropenem. Carbapenemase production was confirmed by Disc diffusion method and then reconfirmation was done by Modified Hodge test. According to study of Girlich D et al., and his co-workers worked on "Value of the Modified Hodge Test for detection of emerging Carbapenemase in Enterobacteriaceae", they found out that the sensitivity to MHT was 77.4% [22]. Among 35 Carbapenemase producing enterobacteriaceae 24 gave positive results and 11 gave negative results. They explained that addition of Zinc improved the sensitivity of MHT [23]. Unlike that, in our study 16 carbapenem resistant strains were isolated by disc diffusion method. Out of 16 resistant strains MHT (without Zinc addition) re-confirmed only 5 positive samples and 11 samples gave false negative results. The sensitivity was found to be 31.25% only.

CONCLUSIONS

In the region of South Punjab there is an increased trend of infections among neonates, especially by *Acinetobacter.* Empirical antibiotics are used as first line treatment. This study showed increased resistant trends of antibiotics. Results of this study are helpful in modifying the empirical treatment. Among multiple antibiotics the resistance to Carbapenem has markedly increased. The production of Carbapenemase has been detected by disc diffusion method and re-confirmed by Modified Hodge test. The results obtained from Modified Hodge test were not satisfactory. So introduction of the antibiotics to the neonates will be done by following the results from disc diffusion method.

Authors Contribution

Conceptualization: RI

Methodology: AJ, AN

Formal analysis: AZ

Writing-review and editing: MSJ, MJ

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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