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Laboratory Survey Of Carbapenem-Resistant Enterobacterales Isolates In Selected Tertiary Hospitals In South-Eastern Nigeria

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ABSTRACT

Objective*: Carbapenems are broad spectrum β-lactam antibiotics that were introduced in response to the emergence of ESBL-producing Gram-negative bacteria. This study aimed at determining the occurence of carbapenem resistant Enterobacterales isolates among patients attending five tertiary hospitals with southeastern region of Nigeria. Methods: A total of 400 Enterobacterales were isolated from different participants. Carbapenem-resistance was detected using Kirby-Bauer disc diffusion method with ertapenem, meropenem, imipenem, and doripenem.* ***Results****: A total of 117 isolates (29.2%) were resistant to the four carbapenems. When compared among the states, the prevalence were 33.6%, 29.1%, 28.8%, 26.9% and 24.7% for Enugu, Ebonyi, Imo, Anambra and Abia states respectively. The highest resistance was observed among P. mirabilis (52.6%) followed by K. oxytoca (35.7%), S. enterica (35.7%) and K. pneumoniae (33.8%).* ***Conclusion****: The overall prevalence was high and this is a cause for concern and urgent need for emergency intervention to forestall widespread emergence of pandrug resistant infections.*

Keywords: Carbapenem, Enterobacterales, Southeast, Nigeria of the mechanical properties of hard dental tissues.

INTRODUCTION

The carbapenems are a group of broad-spectrum beta-lactamase enzymes that possess hydrolytic activities against all cephalosporins, penicillins and carbapenems (Olowo-Okere et al., 2019). However, in the early 1990s the first carbapenem resistant bacteria was isolated in Japan (Iovleva and Doi, 2017). Since then, there have been reports of emergence of carbapenem-resistant organisms in different healthcare settings worldwide (Logan and Weinstein, 2017). The CDC recognized the public health threat and declared that carbapenem resistant organisms require aggressive action. The CDC also reported that up to half of all bloodstream infections caused by carbapenem-resistant Enterobacterales results in death[1-4].

With widespread increase in extended spectrum beta-lactamase producing organisms, there has been increased dependence on carbapenems to effectively treat these infections. However, the development of carbapenem resistance has become a public health malady. They produce difficult to treat infections of all types with associated increase in morbidity, prolonged hospital stay, increased cost of treatment and a mortality rate that is greatly increased to about 50%.

Different mechanisms for carbapenem resistance in Enterobacterales have been reported. They include; reduced outer membrane permeability due to loss of porins, over-expression efflux pumps and production of carbapenemase enzymes. The carbapenemases are grouped according to the Ambler classification as A, B and D. . Other mechanisms for carbapenem resistance include; production of AmpC enzymes and production of extended spectrum beta-lactamases.

Gram-negative organisms are responsible for most clinical infections therefore the emergence of carbapenem resistance among them is of public health significance. Also, there is limited treatment option as approved drugs used against them are fraught with high toxicities and they can be easily disseminated.

There is limited data on the epidemiology of carbapenem resistance among Enterobacterales especially in most sub-Saharan African countries. This study aimed at providing data on the current status of carbapenem-resistant Enterobacterales isolates in selected tertiary hospitals in South-eastern Nigeria.

METHODOLOGY

This Study Area

The south-eastern region of Nigeria lies within the coordinates 5o25'N8o05'E, and is made up of five States; Abia, Anambra, Ebonyi, Enugu and Imo According to the 2006 census, the population of the region was approximately 16,395,555.

Study design

This was a cross – sectional study designed and carried out across five tertiary hospitals in South-eastern Nigeria. Among eleven tertiary hospitals that are located within the region, five of them were selected using simple random sampling technique

Study population

Contaminants The participants were patients who presented with clinical manifestations that suggested the presence of infection(s) with any of the Enterobacterales based on the provisional diagnosis and who had laboratory requests for microscopy, culture and sensitivity. Specimens were collected from the participants as requested in their laboratory forms and the isolates were identified to species level. A total of 400 Enterobacterales isolates were obtained from each of the participants.

Ethical consideration

Ethical approval was obtained from the ministries of health of Abia, Ebonyi, Enugu, Imo and Anambra States. Informed consent was obtained from the participants or from their parents/guardians (for those below 18years).

Sample size

The sample size was calculated using Standard statistical methods and a sample size of 400 was obtained. To determine the number of samples that would be collected from each centre, the probability proportion by size was calculated and the sample size obtained from each centre is presented in (Table 1). The formula used to calculate the sample size to be collected from each centre was; a/b x n; where a= average total samples received by each centre per month b= total number of samples and n= sample size (400)

Table 1 Sample size calculation from the probability proportion by size

S/N	Centre	(a)	Sample size (n)
1	Federal Medical Centre Umuahia, Abia State	900	77.42 (77)
2	Alex Ekwueme Federal University Teaching Hospital, Abakaliki, Ebonyi State	1200	103.22 (103)
3	Enugu State University Teaching Hospital, Parklane, Enugu State	1350	116.13 (116)
4	Imo State University Teaching Hospital, Imo State	600	51.61 (52)
5	Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Anambra State	600	51.61 (52)
	Total (b)	4650	400

Inclusion/Exclusion Criteria

Patients who gave informed consents and in whom Enterobacterales were isolated from their specimens were included in this study. Patients who gave consent but in whom Enterobacterales were not isolated from their specimens were excluded from the study. Also, based on the prescription on the patients' folder and on verbal interview, patients that were on combined antibiotic therapy were excluded from the study.

Specimen Collection and Identification

Enterobacterales were isolated from these various specimens such as urine, sputum, cerebrospinal fluids, stool, blood, semen, wound, high vaginal, ear, throat, urethral and eye swabs. The identification of the isolates was performed using standard microbiological methods described by Cheesbrough (2002) and Forbes et al. (2007) which includes Gram reaction and conventional biochemical tests such as indole, methyl red, Voges-Proskauer, citrate utilization, oxidase, urease, triple sugar iron, and sugar fermentation reactions.

Carbapenem susceptibility and phenotypic screening for resistance

Phenotypic detection of carbapenem resistance was done using Kirby-Bauer disc diffusion method using discs with potency of 10µg each for Ertapenem, Imipenem, Meropenem and Doripenem. The bacterial isolates were suspended in sterile normal saline to match 0.5 McFarland standard. A sterile swabstick was used to inoculate the surface of the Mueller-Hinton agar which had been prepared by following the Manufacturer's instruction and sterilized in an autoclave at 1210C for a holding time of 15 minutes[5].

The antimicrobial discs were placed after the surface of the media had been allowed to dry. The plates were subsequently incubated at 370C for 18 hours. The inhibition zone diameters were measured and the results were interpreted based on guidelines of the European Committee on Antimicrobial Susceptibility Testing version 10 (EUCAST, 2020). Isolates were recorded as carbapenem–resistant if they showed simultaneous resistance to ertapenem, doripenem, meropenem and imipenem.

Data Analysis

Data were analysed with the aid of Statistical package for social sciences (SPSS) version 20.0. Bar charts and pie charts were used for the presentation of some analyses, descriptive analysis, frequency tables and percentages were used for the univariate analysis while Chisquare test was used for the bivariate analysis. P-value <0.05 was considered significant in all analyses.

RESULTS

Enterobacterales isolates were obtained from a total of 400 participants who gave informed consent to be part of this study. Among the participants 208 (52%) were females while 192 (48%) were males. The ratio of the sex was approximately 1:1. The participants ages ranged from 3 years to over 70 years.

The distribution of the specimens obtained for this study as well as the Enterobacterales isolates obtained from each of them is presented in. Urine was the highest specimen collected (137 of 400), followed by wound swab (57), stool (50) and blood (34). The isolates obtained are presented in (Table 2).

Overall, a total of 117 (29.2%) of the Enterobacterales isolates were resistant in all the isolates obtained from the five tertiary hospitals. Among these, the highest resistance was observed among P. mirabilis (52.6%) followed by K. oxytoca (35.7%), S. enterica (35.7%) and K. pneumoniae (33.8%). However, Proteus vulgaris and Y. enterocolytica were fully susceptible to the carbapenems (Table 3). However, there was no significant relationship between the distribution of the isolates and their susceptibility to the carbapenems ($p = 0.346$).

We compared the relationship between the age of the participants and the presence of carbapenem-resistant Enterobacterales isolates but there was no significant relationship ($p = 0.663$). The highest resistance was observed among the age group of 50 – 59 years (37.5%) this was followed by the 30 – 39 age group (32.6%) and the 21 – 29 age group (31.5%). As presented in, the age group with the least frequency of resistance were participants within the 40 -49 age group (17.1%).

The presence of carbapenem resistant isolates were compared among the specimens they were obtained from and there was no significant relationship ($p = 0.123$) as presented in. However, there were more carbapenem-resistant isolates in specimens obtained from eye swab (100%), sputum (50.0%), blood (41.2%), stool (30.0%), throat swab (30.0%) and semen (29.4%).

Resistance to carbapenems was compared among the various states, there was an almost even distribution of carbapenem resistant isolates, hence there was no significant difference in the relationship between the state distributions and resistance to carbapenems. Isolates with the highest resistance were obtained from Enugu (33.6%), while Abia state had isolates with the least resistance to carbapenems (24.7%). Overall, each tertiary hospital had high prevalence of carbapenem-resistant isolates as presented in.

DISCUSSION

This study reports the occurence of carbapenem-resistant Enterobacterales among tertiary hospitals in South-eastern Nigeria. We used strict criteria by recording an isolate as carbapenem-resistant if the isolate was simultaneously resistant to doripenem, meropenem, imipenem and ertapenem. A study which compared phenotypic screening methods for

carbapenem resistance determined that 10µg of ertapenem and imipenem discs had a 100% sensitivity while meropenem disc had 95.7% sensitivity when compared with the E-test and Modified Hodge Test methods (Mohammed et al., 2018). This is comparable to the Kirby-Bauer method for phenotypic detection of carbapenem resistance which was used by this study[6].

There have been consistently increasing reports of high occurrence of antimicrobial resistant isolates in various developing and developed countries. In this study, we investigated the occurrence of carbapenem-resistant Enterobacterales isolates among patients attending five tertiary hospitals in each of the five states that make up the southeastern region of Nigeria. Within these states, various studies have reported high prevalence of Extended-spectrum beta-lactamases producing Enterobacterales across virtually all the tertiary hospitals. We therefore sought to investigate the epidemiology of susceptibility to carbapenems and we found a relatively high prevalence of carbapenem-resistant Enterobacterales. An overall prevalence of 29.2% (117 of 400) was observed among all the samples studied. However, when compared among the respective centres the specimens were collected from, prevalence of 33.6%, 29.1%, 28.8%, 26.9% and 24.7% were observed for Enterobacterales isolates obtained from Enugu state, Ebonyi state, Imo state, Anambra state and Abia States respectively. The relationship between the centres and the presence of carbapenem-resistant Enterobacterales was not statistically significant ($p>0.05$). Because there was almost an even distribution of the resistant isolates among the centres, hence it can be deduced that the spread of these strain of resistant isolates is almost even in all the states within southeastern Nigeria.

The significance of this finding is far reaching considering the dependence placed on this class of antibiotics by clinicians who use them as drugs of last resort for multidrug resistant Enterobacterales isolates.

Among all the centres studied, there has only been one study on carbapenem resistance at Chukwuma Odumegwu Ojukwu University Teaching Hospital by who reported a prevalence of 14.38% of carbapenem-resistant Klebsiella pneumonia and an overall prevalence of 28.21% of carbapenem-resistant Enterobacterales which is comparable to the reports of this study. However there are no other reports of carbapenem-resistant Enterobacterales within other areas studied for comparative assessment of our results[7-8].

Our report is comparable with the prevalence of 28.2% carbapenem resistant Enterobacterales reported by Olowo-Okere et al (2019) in Northern Nigeria. Enwuru et al., (2011) reported a higher prevalence of 36.8% in Southwest Nigeria while Yusuf et al (2015) reported a 34.5% carbapenemase production in Kano, Nigeria. Other studies that reported lower prevalence than this study are Oduyebo et al. (2015) who reported 15.5% prevalence in Lagos and Mohammed et al. (2015) who reported a 10.2% prevalence in Maiduguri. Also, a low prevalence of 7.7% CRE was reported in Southwest Nigeria (Anibijuwon et al., 2018). However a much higher prevalence of 66% carbapenem-resistant Klebsiella pneumonia was reported in Isparta, Turkey.

When the distribution of carbapenem-resistant isolates was compared among the various organisms isolated, the highest resistance was observed among K. oxytoca (10; 35.7%) and S. enterica (5; 35.7%) this was followed by K. pneumoniae (26; 33.8%) and M. morganii (9; 33.3%) and E. cloacae (6; 33.3%). Citrobacter freundii showed the least resistance (14.6%) while Proteus vulgaris and Y. enterocolytica were fully susceptible to the carbapenems. Even though these organisms were highest, the difference amongst all the isolates was not significant ($p = 0.346$), implying that there was no wide difference among them. However, Serratia marcescens and Klebsiella oxytoca were fully susceptible to the carbapenems. Our findings contrast the reports of Olowo-Okere et al. (2019) who found E. coli as the most prevalent resistant isolates. However it reveals the dynamic nature of antimicrobial – resistant genes which can easily spread among various organisms of different genera and families through horizontal gene transfer.

We also found higher occurrence of Y. enterocolytica in extra-intestinal infections (Table 2) in contrast to previous reports. This is a call for further studies to determine if there is a changing epidemiology and pathogenesis in its infection within the study area[9-10].

The presence of carbapenem-resistant Enterobacterales signals an urgent call for the strengthening of antimicrobial resistance surveillance programs. Across Nigeria, there is a dearth of data on antimicrobial resistance among isolates from hospitals and almost an absent emergency reporting channel when multidrug resistant isolates are detected in clinical laboratories. There is an abundance of last resort antibiotics which are sold over the counter without control. Worst still is the practice of empirical therapy by clinicians as well as self medication and abuse of antibiotics. Together, the factors that trigger the development and spread of antimicrobial resistance genes are numerous and multifaceted. However, there can be effective control with necessary actions. The need for strict control of antibiotics cannot be over-emphasized. Also is the urgent need for the institution and implementation of infection-control policies and programs. The high prevalence of carbapenem-resistant isolates in the various centres studied and the reports from other geographic regions of Nigeria begs the question; are we entering the post-antibiotic era?[11-13].

CONCLUSION

This study has demonstrated a relatively high prevalence of carbapenem-resistant Enterobacterales isolates among patients attending five selected tertiary hospitals in South-eastern Nigeria. This is a call for further studies to determine the resistance mechanisms and signals the need for concerted efforts towards effective antimicrobial surveillance programs and infection-control prevention measures to reduce the rate of spread of these resistant bacteria to the barest minimum. The rapid spread and distribution of carbapenem-resistant isolates across the various centres studied also calls for more efforts to be geared towards the production of new antibiotics as a sufficient response to the increasing trend of bacteria which are expensively antibiotic resistant taking into consideration that carbapenems are currently reserved as the drugs of last resort for infections caused by multidrug resistant bacteria[14-15].

DECLARATIONS

Limitation

Due to paucity of funds, this study did not detect the mechanism (s) of resistance to the carbapenems.

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Declaration of conflicts of interest

The authors declare no conflicts of interest.

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