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A study of the extended-spectrum beta-lactamases and ambler class C beta-lactamases in environmental samples

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Abstract
Extended-spectrum beta-lactamases (ESBLs) and Ambler class C lactamases (Amp C) have emerged to provide bacteria with formidable resistance to modern drugs. The current study aimed to examine the presence of ESBL and Amp C producing isolates among environmental samples in the rural communities of Enugu State, Nigeria. A total of 42 isolates were screened and observed for the presence of ESBL and Amp C producing isolates. The double-disc approximation method was used, the production of either of the two enzymes was detected by observing zones of inhibition in diameters. The result showed the presence of ESBL and Amp C in the samples tested. The work reveals that Escherichia coli (8) is the significant ESBL producer, followed by Pseudomonas aeruginosa and Klebsiella pneumoniae (6). The observed synergistic effect in the zone of inhibition between the disc of Ceftazidime and Amoxicillin-clavulanic acid indicates the presence of ESBL. In contrast, Amp C's presence was characterized by no observation of synergism in the zone of inhibition between the disc of Ceftazidime and Amoxicillin-clavulanic acid disc. This study recommends adequate and appropriate detection and monitoring of ESBL and Amp C producing enzymes in rural communities.

Keywords: ESBL, amp C, antibiotics, clinical, environment

Introduction

Different antibiotics in combating the growth and spread of microorganisms and infections have been widely adopted across all cultures and societies (Tanko, Bolaji, Olayinka, & Olayinka, 2020). Perhaps, the rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria has been recognized as a significant public health concern (Bennani *et al*., 2020; Buchy *et al*., 2020; Christaki *et al*., 2020; Dadgostar, 2019; Lomazzi *et al*., 2019; Palma *et al*., 2020; Qu *et al*., 2019; Reed *et al*., 2019; Taneja & Sharma, 2019; Vidovic & Vidovic, 2020; WHO, 2020) [4, 5, 6, 7, 14, 28, 36, 38, 40] Antimicrobial resistance has continued to threaten the progress in health and the attainment of sustainable health development goals. The resistance to beta-lactam antibiotics is an increasing problem worldwide, and beta-lactamase production is the most common drug resistance mechanism (Gupta, 2007) [9].

Extended-spectrum beta-lactamases, most commonly found in Klebsiella pneumoniae and Escherichia coli, have increased markedly in the past decade, particularly in the intensive care unit setting (Patterson, 2003) [23]. The prevalence of extended-spectrum beta-lactamases (ESBLs) in Gram-negative bacteria is a significant challenge for clinicians (Sutton, 2014) [35], primarily due to the difficulty managing these organisms. Ghafourian *et al*. (2014) [8] explained ESBLs as enzymes formed by bacteria that have the potential to hydrolyze extended-spectrum cephalosporin. Evidence suggests that ESBLs are very active against beta-lactam antibiotics like ceftazidime, ceftriaxone, cefotaxime, and oxyimino monobactam. The threat of ESBLs to organisms occurs in varying directions. For instance, its threat arises directly from their vast substrate potentials in penicillin, cephalosporins, and monobactams. The dissemination and threat of ESBLs have been widely investigated (Ampaire *et al*., 2017; Lee *et al*., 2016; Lona-Reyes *et al*., 2019; Manoharan *et al*., 2016; Mohanty *et al*., 2010; Ramazanzadeh *et al*., 2016; Saeed *et al*., 2020; Shaikh *et al*., 2015; Smail & AL-Otrachi, 2020; Tarazi *et al*., 2020) [2, 13, 15, 17, 18, 25, 29, 37].

Furthermore, the detection of Amp C-mediated resistance in Gram-negative organisms poses a problem due to misleading results in phenotypic tests (Akujobi *et al*., 2011; Hemalatha *et al*., 2007; Vijayvergia *et al*., 2013) [1, 12, 39]. Amp C's observation is essential due to the unstandardized method of the phenotypic tests that frequently lead to ambiguity in interpreting results (Shanthi *et al*., 2012) [31]. Amp C beta-lactamases confer resistance to a wide variety of antibiotics and pose diagnostic and therapeutic challenges (Handa *et al*., 2013; Hassan *et al*., 2011) [10, 11]. Anusuya and Ramachander (2015) [3] stated that failure to detect ESBL and Amp C enzymes earlier in certain clinical and environmental samples had been implicated in its widespread. The primary reason for detecting these enzymes is their prevalence and associated therapeutic failure, morbidity, and mortality (Onyedibe *et al*., 2015) [21]. Thus, the present study aimed to determine the occurrence of these enzymes in the clinical and environmental samples in the Enugu state's rural communities.

Materials and Methods

The study was carried out in rural areas of Enugu State, Nigeria, between August 2019 and November 2019. A total of 42 environmental samples comprising soil, water, and food were obtained from the rural communities' general surroundings.

Detection of EBSL and Amp C beta-lactamases.

The detection of the enzymes from the obtained

environmental isolates was done according to the method outline by Rao and Harle (2018) [26].

Result

A total of 42 environmental and clinical samples were tested for the presence and absence of ESBL and Amp C. The result showed that 21 isolates were positive for ESBL, 13 isolates tested positive for Amp C, while eight isolates showed negative for the enzymes. The observed synergistic effect in the zone of inhibition between the disc of Ceftazidime or Ceftriozone and Amoxicillin-clavulanic acid indicates the presence of ESBL. In contrast, the presence of AmpC was characterized by no observation of synergism in the zone of inhibition between the disc of Ceftazidime or Ceftriozone and Amoxicillin-clavulanic acid disc. The absence of ESBL and Amp C enzymes was indicated by observing a relatively larger zone of inhibition compared with the synergistic zone of inhibition of the above and more significant than that observed when the Cefuroxime disc was placed alone.

Table 1: Table showing the presence and absence of ESBL and Amp C enzymes in both clinical and environmental samples

Isolates	Number Tested	ESBL	Amp C	Absence of both enzymes
E. coli	8	5	2	2
Klebsiella spp	6	2	3	2
Pseudomonas spp	6	3	2	-
Proteus spp	3	2	2	-
Streptococcus Aureus	5	2	-	2
Staphylococcus Aureus	4	2	1	-
Salmonella spp	5	3	2	1
Citrobacter spp	3	-	1	1
Enterobacter spp	2	2	-	Nil
Total	42	21	13	8

Discussion

The present study aimed to investigate the occurrence of extended-spectrum beta-lactamases and ambler class C beta-lactamases in environmental samples. The samples' screening showed the presence of ESBL and Amp C producing isolates in the environmental samples tested. The result supports previous studies indicating that ESBL and Amp C producing isolates is a common microorganism in our society (Malik *et al*., 2015; Muzslay *et al*., n. d.; Nuangmek *et al*., 2018; Rastogi *et al*., 2010; Sheela *et al*., 2014; Sudarwanto *et al*., 2017). Thus, their existence has been identified as a public health challenge. The isolated ESBL in the study is 21 higher than the Amp C, signifying a higher rate of ESBL. The observed rate of ESBL in the study could be attributed to the imprudent usage of extended-spectrum beta-lactamase in our environment, which could be avoided by adopting appropriate infection control measures. More so, the presence of Amp C beta-lactamases in environmental samples such as soil and plants indicates urgency in the level of detection of their resistance mechanism. Ecological source of Amp C mediated resistance suggests the need for proper screening of isolates from community-acquired plasmid-encoded Amp C mediated resistance within the hospital.

Conclusion

The study was carried out to investigate the prevalence of ESBL and Amp C extended-spectrum beta-lactamases production in environmental samples. The result showed the presence of ESBL and Amp C in the samples tested. The work provides valuable data for the prevention and spread of these enzymes. The present study reveals Escherichia coli (8) as the significant ESBL producer, followed by Pseudomonas aeruginosa and Klebsiella pneumoniae (6). The major limitation of the current study is the small number of samples tested. However, the study contributes to the literature by revealing these enzymes' occurrence in the rural communities in the Enugu state of Nigeria. It is recommended that adequate and proper screening of ESBL and AmpC producing enzymes within the communities.

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