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RESEARCH ARTICLE

The Prevalence of Extended-Spectrum Beta-Lactamases and Ambler Class C Beta-Lactamases in Enugu and Ebonyi States of Nigeria

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Abstract

Over the years, antibiotics have been among the primary drugs used to treat or prevent infections in animals and humans. Over the years, ESBL and AmpC producing isolates have become common microorganisms in medical institutions. They have contributed immensely to the clinical challenges caused by nosocomial infections with minimal therapeutic options. The present study investigated the prevalence of extended-spectrum beta-lactamases and Ambler class C beta-lactamases in the Enugu and Ebonyi States of Nigeria. A total of fifty isolates comprising 27 clinical and 23 environmental isolates were screened and observed for the presence of ESBL and AmpC producing isolates. The production of either of the two enzymes was detected by observing zones of inhibition in diameter. The findings showed the presence of ESBL and AmpC producing isolates in the clinical and environmental samples tested. The study concludes that ESBL and AmpC beta-lactamases production by resistant bacterial isolates (especially the Gram-negatives) remain the prevalent mechanism in bacterial resistance to beta-lactam drugs. The study recommends that adequate and appropriate detection and monitoring of ESBL and AmpC producing enzymes within the communities.

Introduction: -

Over the years, antibiotics have been among the primary drugs used to treat or prevent infections in animals and humans (Tanko et al., 2020). Perhaps, the rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria that have been observed over the last two decades is widely accepted to be one of the major problems of human medicine today (Gniadkowski, 2001). Antimicrobial resistance is a global problem that threatens progress in health and the achievement of sustainable development goals. Since their discovery in Germany in 1983 and 1988 for ESBL and AmpC, these enzymes (beta-lactamases) have become a worldwide problem (Bradford, 2001).

Extended-spectrum beta-lactamases (ESBLs) are defined as enzymes produced by certain bacteria that can hydrolyze extended-spectrum cephalosporin (Ghafourian et al., 2015). ESBLs are often plasmid-mediated, and most are members of the TEM and SHV families of enzymes (Mushtak, Kubaisy, & Ani, 2011). They have proven to very active against beta-lactam antibiotics like ceftazidime, ceftriaxone, cefotaxime, and oxyimino monobactam (Bradford, 2001; Paterson & Bonomo,2005). The threat of ESBLs to organisms occurs in varying directions. Its threat arises directly from their vast substrate potentials in penicillin, cephalosporins, and monobactams. It has been observed worldwide that there is high dissemination of extended-spectrum beta-lactamase (ESBL)-producing,

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methicillin- and carbapenem-resistant bacteria (Tanko et al., 2020). On the other hand, Ambler class C (Amp C) beta-lactamases are cephalosporinanses that are poorly inhibited by clavulanic acid and can be differentiated from ESBLs ability to hydrolyze cephamycins as well as other extended-spectrum cephalosporins. (Singhal et al., 2005). They are believed to have originated from the chromosomes of Enterobacter, Citrobacter, and Pseudomonas spp (Philip et al., 2000). The rapid and irrepressible increase in antimicrobial resistance of pathogenic bacteria that have been observed over the last two decades is widely accepted to be one of the major problems of human medicine today (Winokur et al., 2000).

Detecting and reporting isolates producing plasmid-mediated AmpC beta-lactamases are more challenging than those associated with ESBLs (Mushtak, Kubaisy, & Al-Ani, 2011). The detection of ESBLs is a challenge for routine clinical microbiology laboratories in resource-limited settings. The detection of a decrease in oxyimino-cephalosporins' susceptibility is not sufficiently sensitive to detect all ESBL-producing strains (Harwalkar, et al., 2013). The inability to see these enzymes earlier in certain clinical samples has been implicated in its widespread. The indiscriminate and extensive use of newer antimicrobial drugs has resulted in the emergence and rapid dissemination of resistant bacterial species capable of producing these enzymes either chromosomally or as plasmid-mediated (Gniadkowski, 2001). The concern for the detection and occurrence of ESBLs and AmpC β-lactamases is due mainly to the ubiquitous prevalence in nosocomial infections intensive care unit (ICU) and its association with therapeutic failure, especially in life-threatening conditions (Mushtak, Kubaisy, & Al-Ani, 2011).There is an increasing prevalence of ESBLs producers, and the ESBLs producer strains are causing higher levels of morbidity, mortality, and healthcare-associated costs (Ghafourian, Sadeghifard, Soheili, & Sekawi, 2015). Undoubtedly, if immediate and proper attention is no given to the growing health problems caused by these enzymes, then achieving a successful therapeutic result will most be a mirage.

Beta-lactamases (ESMLs and AmpC) are abundant in our environments, such as water, soil, and domestic animals (Rasheed et al., 2007). Plasmid-encoded AmpC beta-lactamases, for instance, have been found in isolates from livestock such as swine and cattle and companion animals (Nancy, 2003). Of course, their presence in these environments causes numerous infections such as diarrhea and gastroenteritis (Rasheed et al., 2007). More ominous is that recent research has indicated the propensity for these bacterial strains to establish a long-term resistance in the environment (Bonnet, 2004).

The Present Study

Aerobic gram-negative bacilli-triggered infections are commonly found among patients that are hospitalized. Over the years, there has been a notable increase in bacterial infections such as bacteremia and nosocomial pneumonia, and the majority of these infections are contracted in hospitals. These infections are associated with high rates of mortality. For instance, sepsis is one of the most common causes of death among patients in the intensive care unit. The emergence of bacteria that contain the extended-spectrum beta-lactamases (ESBLs) and the Ambler class C lactamases (AmpC) has compounded this problem and has become a global concern. Whereas many studies on the detection of ESBL in Gram-negative bacteria in Nigeria, there are regions where the cases are still under-reported and the prevalence still unclear (Tanko et al., 2020). This study aimed to determine the occurrence of these enzymes in the clinical setting and examine the presence of ESBL and AmpC producing isolates among environmental samples in Ebonyi and Enugu state's rural areas.

Materials and Methods: -

Collection of samples

Clinical and environmental samples were collected from clinics, laboratories, hospitals, and the general environment. Samples such as stool, urine, sputum, throat swab, midstream urine, pus, aspirate, and semen made up the clinical samples whereas, the environmental samples included soil, water, and food. The specimens were appropriately categorized and stored accordingly.

Preparation of culture media

A total of fourteen grams of the nutrient agar powder was weighed and dissolved in 500ml of distilled water. The mixture was allowed to soak for 10 minutes and was afterward autoclaved at 121^{o}C (15p.si) for 15 minutes. The nutrient agar was allowed to cool to 45^{o}C, after which 15-20ml each was dispensed aseptically into Petri dishes. Also, 5.2g of the Conkey agar powder was weighed and dissolved in 100ml of distilled water. The mixture was allowed to soak for 10minutes and afterward autoclaved for 15 minutes at 121^{o}C (15p. si). The agar was allowed to cool to 45^{o}C before dispensing 15-20ml aseptically into Petri dishes. The sample was inoculated on either Mac-

Conkey or nutrient agar using a sterile loop. The plates were incubated at 37ºC for 24 hours, after which the bacterial colonies were observed from isolation based on their different morphologies, characteristics, and color. The isolates were inoculated in nutrient agar slants using sterile.

Detection of EBSL and AmpC beta-lactamases using double disc approximation method.

Pure isolates obtained from the clinical and environmental samples were used, and suspension of these isolates was prepared and inoculated on nutrient agar using a sterile loop. Amoxicillin- clavulanic acid disc was placed towards the center of each plate. Ceftazidime disc was put 15-20mm away to the left of the Amoxicillin- clavulanic acid disc. Ceftriaxone disc was placed 15-20mm away to the right. Ciprofloxacin disc was placed 15-20mm directly above, while ofloxacin disc was established 15-20mm directly below Amoxicillin Clavulanic acid disc so that they are paced 90^0c apart from the center disc. Cefuroxime disc was placed separately from the rest to the bottom left; Amoxil disc to the bottom right. And Cephalexin disc set individually at the upper right. The plates were incubated at 35ºC for 24hrs, and the zone of inhibition in diameter of each plate was observed and read.

Result: -

A total of fifty isolates comprising 27 clinical and 23 environmental isolates were screened and observed for the presence of ESBL and AmpC producing isolates. The production of either of the two enzymes was detected by observing zones of inhibition in diameter. The inhibition was a result of the effect of the drug used on different clinical isolates. The observed synergistic impact in the zone of inhibition between the disc of Ceftazidime or Ceftriaxone 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 Ceftriaxone and Amoxicillin-clavulanic acid disc. The absence of ESBL and AmpC 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.

The table below shows the potency of the disc used (µg)

Antibiotic Disc	Formular	Potency
Amoxicillin-clavulanic acid	AMC	20 µg
Ceftazidime	TAZ	30 µg
Ceftriaxone	TRI	30 µg
Cefuroxime	URO	30 µg
Cefalexin	CEF	30 µg
Ciprofloxacin	CIP	10 µg
Ofloxacin	OFX	10 µg
Amoxil	AML	10 µg

The table below shows the presence and absence of ESBL and AmpC enzymes in both clinical and environmental samples

Isolates	Number Tested	ESBL	AmpC	Absence of both enzymes
E. coli	11	7	1	3
Klebsiella spp	8	3	4	1
Pseudomonas spp	7	4	3	Nil
Proteus spp	6	3	3	Nil
Streptococcus Aureus	6	3	Nil	3
Staphylococcus Aureus	5	4	1	Nil
Salmonella spp	4	2	1	1
Citrobacter spp	2	Nil	1	1
Enterobacter spp	1	1	Nil	Nil
Total	50	27	14	9

The above table shows that 50 environmental and clinical samples were tested for the presence and absence of ESBL and AmpC. It was observed that 27 isolates were positive for ESBL, 14 isolates tested positive for AmpC, while nine isolates were reported the absence of the enzyme.

The table below shows the presence and absence of ESBL and AmpC enzymes in environmental samples

Isolates	Number tested	ESBL	AmpC	Absence of both enzymes
E. coli	5	3	Nil	1
Klebsiella spp	3	1	1	1
Pseudomonas spp	3	1	2	Nil
Proteus spp	3	2	1	Nil
Staphylococcus Aureus	2	2	Nil	Nil
Streptococcus Aureus	3	1	Nil	2
Salmonella spp	2	2	Nil	Nil
Citrobacter spp	2	Nil	2	Nil
Total	23	12	6	4

The table above shows the occurrence of ESBL and AmpC beta-lactamases in environmental samples. It indicates that from the total number of ecological isolates tested, 52.2% representing 12 isolates tested positive for ESBL, 26.1% tested positive for AmpC, while 21.8% tested positive for the absence of both ESBL and AmpC.

The table below shows the presence and absence of ESBL and AmpC enzymes in clinical samples.

Isolates	Number tested	ESBL	AmpC	Absence of both enzymes
E. coli	6	4	1	1
Klebsiella spp	5	2	3	Nil
Pseudomonas spp	4	3	1	Nil
Proteus spp	3	1	2	Nil
Staphylococcus Aureus	3	2	1	Nil
Streptococcus Aureus	3	2	Nil	1
Salmonella spp	2	Nil	1	1
Enterobacter spp	1	1	Nil	Nil
Total	27	15	9	3

The table above shows the occurrence of ESBL and AmpC beta-lactamases in environmental samples. It indicates that from the total number of ecological isolates tested, 55.6% representing 15 isolates tested positive for ESBL, 33.3% tested positive for AmpC, while 11.1% tested positive for the absence of both ESBL and AmpC.

Discussion: -

The present study's findings showed the presence of ESBL and AmpC producing isolates in the clinical and environmental samples tested. Over the years, ESBL and AmpC producing isolates have become common microorganisms in medical institutions. Perhaps, they have contributed immensely to the clinical challenges caused by nosocomial infections with minimal therapeutic options. Their extraordinary rapid rate of reproduction and the possibility that their enzyme coding sequence can be located on high copy number plasmids give them a further advantage of having an extremely high probability of being produced in the clinical setting (Nancy, 2003). Though ESBL is more frequently encountered in clinical samples than the AmpC, recent studies have shown that ESBL-producing strains have spread into the communities (Mitchel et al., 2004). This is demonstrated by the growing incidence of ESBL in Salmonella spp, which portrays the dangers of spread among pathogens circulating in livestock and the community.

Most of the AmpC beta-lactamases were found in the clinical specimens, suggesting that AmpC-harboring isolates are primarily restricted to hospitalized patients. However, the presence of AmpC beta-lactamases in environmental samples such as livestock (e.g., swine, cattle, and dog), soil, and plants indicate urgency in the level of detection of

their resistance mechanism. A community-based source for AmpC mediated resistance suggests that hospital-based clinical laboratories should be screening isolates from community-acquired plasmid-encoded AmpC mediated resistance within the hospital. Though E. coli, Klebsiella pneumonia, and Proteus spp are the species in the family of Enterobacteriaceae most commonly associated with these enzyme productions, only a few studies have honestly assessed the occurrences AmpC beta-lactamases among these organisms (Vikas & Narendra, 2003). It is of therapeutic interest for s clinical laboratory to distinguish between these beta-lactamases.

Conclusion: -

The double-disc approximation method was applied for this study. It indicates that ESBLs are more frequently encountered in both clinical and environmental samples than AmpC beta-lactamases. The variation can be attributed to certain environmental conditions such as PH, temperature, and enzyme concentration, making it more suitable for the production of ESBL than AmpC in these samples. The current study concludes that ESBL and AmpC beta-lactamases production by resistant bacterial isolates (especially the Gram-negatives) remain the prevalent mechanism in bacterial resistance to beta-lactam drugs (Araj & Kanj, 2000). This resistance mechanism will continue to be a worldwide scourge in clinical medicine if immediate action is not taken. Increasing detection of Klebsiella spp, E. coli, and Proteus spp and other members of the Enterobacteriaceae that produce ESBLs are most frequently encountered in both clinical and environmental isolates when compared with AmpC. This observation suggests that environmental factors like PH concentration and temperature in some clinical and environmental samples are suitable for the production of ESBLs but not suitable for AmpC production, hence the variation in the occurrence rate. This study recommends that adequate and appropriate detection and monitoring of ESBL and AmpC producing enzymes within the communities. Type identification of ESBL and AmpC is highly required to aid physician's prescription strategies.

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