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AmpC Beta-Lactamase-Producing Escherichia coli: Prevalence and Characterization in Livestock: A Risk to the Safety of Antibiotics for Human and Animal Health

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RESEARCH ARTICLE

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Abstract: The third generation extended spectrum cephalosporins and cephamycins are hydrolyzed by bacterial enzymes called ampC beta-lactamases, which leads to resistance to these antibiotic classes and poses a severe danger to the present supply of antibiotics used in both human and animal medicine. This study looked at the identification of AmpC beta-lactamase-producing E. coli in a few popular livestock species. 196 aseptic faecal samples from pigs, cattle, fowl, and goats in various locations throughout Uyo Metropolis were collected and placed into sterile universal containers.

Following the inoculation of the samples onto macConkey agar using the streak plate technique, the samples were incubated at 37 °C for 18 to 24 hours, during which time the growth was detected using conventional identification techniques. The agar disc diffusion method was utilised to ascertain the susceptibility profile of every discovered E. coliisolate to specific antibiotics. Using the modified double disc synergy test, which detects ESBL producers, resistant E. coli isolates to third and fourth generation cephalosporins were screened, and the modified disc test was used to manufacture AmpC beta-lactamase. The outcome demonstrates that, of the 123E. coliisolates, 55.68 percent were probable ESBL producers and 30.68 percent were verified to be AmpC producers. Pig and Goat contributed the lowest percentages (3.23 percent) and the greatest percentage (5.37 percent) correspondingly.

The study's findings demonstrate that AmpC beta-lactamase-producing E. coli was present in every animal test group. Consequently, there is an urgent need to increase the surveillance of antibiotic usage and antibiotic-resistant microorganisms in farm animals. Implementing biosecurity and hygiene measures in the cattle breeding industry has a positive impact on preventing the spread of antibiotic resistance.

Keywords: cattle, and detection. Escherichia coli, AmpC,

1. INTRODUCTION

Microorganisms developing antibiotic resistance is a serious issue for the human and cattle industries. The continual exposure of bacterial strains to various β-lactam antibiotics has resulted in the production of specific enzymes in these bacteria being mutated and dynamic, which in turn has increased their activity against the newly created β-lactam antibiotics. The enzymes in question are referred to as extended-spectrum β-lactamases (ESBL)[1,2]. The over-expression of a different kind of enzyme that preferentially hydrolyzes cephalosporins and cephamycins with a limited, broad, and extended range exacerbates this issue even further. Additionally, they have the ability to withstand the inhibition caused by clavulanate, sulbactam, and tazobactam. We refer to these enzymes as AmpC β-lactamases. The chromosomes

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of many Enterobacteriaceae and a few other species include AmpC β-lactamases, which are clinically significant cephalosporinases that cause resistance to cephalothin, cefazolin, cefoxitin, most penicillins, and combinations of β-lactamase inhibitor/β-lactam. AmpCenzymes can be inducible and highly expressed in a variety of bacteria.

Currently, a lot of clinical laboratories test Escherichia coli for the production of extended-spectrum β-lactamases (ESBLs), but they don't try to find plasmid-mediated AmpC β-lactamases. This is likely because the available phenotypic tests are either difficult to use, subjective, or require reagents that aren't easily obtained[3, 4]. Within the Nigerian livestock industry, the presence of β-lactamase-producing Escherichia coli has been well acknowledged in veterinary medicine, serving as causal agents for mastitis in dairy cattle, for example[5]. Due to their frequent encounters in routine disease diagnosis brought for confirmation diagnosis in microbiological diagnostic departments of several tertiary veterinary teaching hospitals, this problem is becoming increasingly widespread[5]. Merely a handful of research works in South-South Nigeria have examined the frequency of β-lactamase-producing bacteria in animals. It is still mostly unknown how common zoonotic transfer is from livestock to people who have close contact with these animals, but some research has linked farm workers to the transmission of ESBL-producing E. coli or ESBL genes from poultry or pigs[6, 7]. In addition to this straight

2.MATERIALS AND METHODS

2.1 Gathering of Examples

Between January and March of 2017, 196 fresh faeces samples were aseptically and randomly collected from cattle, chicken, goats, and swine into sterile universal containers from various locations within Uyo Metropolis in Akwa Ibom State, Nigeria. They were brought to the University of Uyo Teaching Hospital's Medical Microbiology and Parasitology Laboratory and kept at 4 °C until they were needed for processing. 2.2 Sampling Processing Before being inoculated using the streak plate method on MacConkey agar (Oxoid, UK), one gramme of the faecal samples was emulsified in five millilitres of sterile saline. The samples were then incubated at 37ºC for eighteen to twenty-four hours, after which they were checked for growth. Different smooth, glossy, rose-pink lactose-fermenting colonies thought to be E. coli were chosen based on the growth pattern of the organisms seen on the agar plate, and they were then subjected to identification methods in accordance with standard Cowan taxonomic identification schemes[9].

2.3 Establishing the Profile of Antimicrobial Susceptibility

TThe agar disc diffusion method was utilised to examine the susceptibility profile of the detected E. coli isolates to specific antibiotics, in accordance with the advice of the Clinical and Laboratory Standards Institute, CLSI [10]. Cefotaxime (30µg), cefpodoxime (30µg), ceftrioxone (30µg), ceftazidime (30µg), cefepime (30µg), cephoxitin (30µg), gentamicin (10µg), ciprofloxacin (5µg), norfloxacin (10µg), nitrofurantoin (100µg),cotrimoxazole (25µg), and imipenem (10µg) were the antibiotics that were used. The source of all the antibiotic discs was Oxoid. Five millilitres of sterile peptone water were used to suspend recently produced bacteria in order to create the bacterial inoculum. After the suspension was adjusted to reach a turbidity comparable to 0.5 McFarland turbidity standards, Mueller Hinton (MH) agar plates were filled with the inoculated peptone water, and the excess was drained away. Using sterile forceps, the plates were allowed to dry before the proper antibiotic discs were aseptically placed on the agar plate surface. After that, the plates were incubated for 18 to 24 hours at 37 °C. The Kirby Bauer test method was utilised to estimate the diameter of the zone of inhibition, as

detailed by

2.3.1Chromogenicagarculture

A small number of E. Coli isolates that showed resistance to third- and fourth-generation cephalosporins were homogenised in 1 ml of sterile physiological saline (0.85%). 50 µlaliquots of the resulting suspension were then inoculated onto chromogenic ESBL-Bx agar, which was made from the dehydrated medium in accordance with the manufacturer's instructions and incubated in ambient air at 37 °C for 18 to 24 hours. Following the ideal incubation period, ESBL generation is indicated by a certain hue that is amplified by the chromogenic media.

2.4Extended SpectrumBeta-Lactamase Production Screening

The modified double disc synergy test was used to further screen the E. coli isolates that showed ESBL synthesis and resistance to third and fourth generation cephalosporins in order to identify ESBL producers.

2.4.1 Modifieddouble disc synergy test

Amoxicillin-clavulanate (20/10µg) disc was used for this, in addition to four cephalosporins: cefotaxime, ceftriaxone, cefpodoxime, and cefepime from the third and fourth generations. In a nutshell, the test isolates were grown on an agar Mueller-Hinton plate. A disc containing 20/10µg of amoxicillin-clavulanate was positioned in the middle of the plate. The cefotaxime, ceftriaxone, and cefpodoxime discs were positioned 15 mm apart, and the cefepime disc was positioned 20 mm apart, centre to centre, between the amoxicillin-clavulanate disc (Paterson and Bonomo). Any change or expansion of the inhibitory zone in the direction of the amoxicillin-clavulanate disc was interpreted as evidence of the formation of ESBLs.

2.4.2 Boost Production of Enzymes

In the modified double disc synergy test (MDDST), isolates that showed a strong synergistic impact with just cefepime were examined further for AmpC enzyme production using the modified disc test. The test involves permeabilizing a bacterial cell with Tris-EDTA and releasing β-lactamases into the external environment, as described in Umoet al.;AJOB,5(3):1-7, 2018; Article no.AJOB.405164. The modification made by Kauret al.[12] was carried out. In summary, sterile plain 6mm discs were punched out of Whatmann filter paper, and 20µl of a 1:1 saline and Tris-EDTA combination was applied to the discs to create the AmpCdisks. After being left to dry, the discs were kept in storage at 2 to 8 °C. On a Mueller-Hinton agar plate, a suspension of standard E. coli ATCC25922 equals to 0.5 McFarland turbidity standards was made and inoculated.

A 30µg cephoxitin disc was positioned on the agar surface that had been infected. The AmpC discs that were manufactured were rehydrated with 20µl of saline before to being inoculated with several colonies of the testisolates. This was set up next to the cephoxitin disc, and the plates were incubated for 18 to 24 hours at 37 °C. The plates were inspected for either an indentation or flattening of the zone of inhibition, which is a positive result indicating the enzymatic inactivation of cephoxitin, or for the lack of distortion, which is a negative result indicating no significant inactivation of cephoxitin.

3. RESULTS

Four distinct animal species provided a total of 196 faecal samples that were collected and tested. Of these, 23.98 percent came from cattle, 26.53 percent from chicken and goat, and 22.96 percent from pigs (Table 1). Twenty-three E. coliisolates were isolated from all of the stool samples that were examined. Samples from poultry and cattle produced 13.27 percent and 18.87 percent, respectively, samples from goats produced 16.33 percent, and samples from pigs produced 14.29 percent, for a total output of 62.76 percent. Seventy-one.5% of the 123E. coli

isolates that were collected showed resistance to third- and fourth-generation cephalosporins. Further testing for ESBL production using the Modified Double Disc Synergy Test (MDDST) revealed that 55.68 percent of the sample were ESBL producers, of which 12.5 percent tested positive for AmpC co-production using the AmpCdisc test, and the remaining 43.18 percent were exclusively ESBL producers. But among the isolates taken from Pig, the greatest percentage of 4.55 percent was seen.

4. DISCUSSION

The use of β-lactam treatment is seriously threatened by Extended Spectrum Beta-Lactamases (ESBLs). There is a growing body of evidence indicating that Enterobacteriaceae, which are found in both people and animals, are becoming resistant to β-lactam antibiotics. Many of these strains have been incorrectly claimed to be responsive to the widely used broad-spectrum β-lactam antibiotics because they are difficult to identify by the current clinical procedures available in most laboratories[13]. All of the tested animals in the group have ESBL-producing E. coli, according to the study's findings.

An investigation carried out in Germany looked for ESBL-producing E. coli in various dairy, beef, and mixed farms (both dairy and beef). The findings revealed a significant incidence of various ESBL subtypes [14]. Spain conducted the first reports of ESBL-producing bacteria from chicken. It was discovered that E. coli strains isolated from faecal samples of both sick and healthy poultry carried different kinds of ESBL genes[15].

Similar to this, antibiotic resistance in commensal Enterobacteriaceae from pigs was also verified in some Danish farms, where some strains of E. coli that produced ESBL were found in the faeces of the pigs[16]. Generally speaking, birds and animals are possible carriers of microorganisms that are resistant to drugs. This is due to the fact that plasmids, which are frequently used to carry ESBL-encoding genes, are easily transferred between isolates and carry additional resistance determinants for other classes of antimicrobial agents, primarily fluoroquinolones, aminoglycosides, and sulfonamides, which contribute to the multidrug-resistant phenotype.

Table 1: Escherichia coli Isolate Sources

Source of sample	No. of samples collected	No.(%) of *E. coli* isolated
Cattle	35	18(13.27)
Chicken	40	29(18.87)
Goat	40	25(16.33)
Pig	33	21(14.29)
Total	**196**	**123(62.76)**

Table 2. ESBL and *AmpC* producing *E. coli* Isolates (n=88)

Source of sample	No.(%) of potential ESBL producing isolates	No.(%) of ESBL & AmpC producing isolates	No.(%) of only ESBL producing isolates
Cattle	10(11.36)	2(2.27)	8(9.09)
Chicken	13(14.78)	3(3.41)	10(11.36)
Goat	10(11.36)	2(2.27)	6(6.82)
Pig	16(18.18)	4(4.55)	14(15.91)
Total	**49 (55.68)**		

to make AmpC, and 55.68 percent were ESBL producers. According to Kaur et al. [12], 63.4 percent of ESBL producers were AmpC producers. This is consistent with their findings. According to this study, among the isolates taken from pigs, AmpC production has the highest prevalence (4.55 percent). As previously demonstrated by Jorgensen et al. [16], who in their earlier study confirmed that the use of beta-lactam antibiotics, especially cephalosporins, might be one of the factors for the selection of ESBL/AmpC-producing bacteria in pigs, this may be connected to the use of antibiotics in pig production. Also Carattoli et al. [17]found that the intestinal flora of pigs is selective for E. coli bacteria that produce beta-lactam antibiotics, such as amoxicillin, when these antibiotics are utilised in pig production.

Additionally, the selection of beta-lactamase genes may be influenced by antibiotics other than beta-lactams. It is imperative that ESBL testing be done on Enterobacteria cae species that produce amp C. When AmpC and ESBL are present in gram-negative bacteria, the DDST might not produce a positive result because the AmpC type of β-lactamase prevents clavulanate from working. Therefore, it masks the combined benefits of

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clavulanic acid and the third-generation cephalosporins that are employed. One possible solution to the challenge of ESBL detection in the presence of AmpC is to employ tazobactam or sulbactam as inhibitors of the ESBL detection tests with these organisms. These compounds are less likely to induce the β-lactamases of AmpC, making them the preferred inhibitors. Another option is to test cefepime as an ESBL detection agent.

The regulators of the antibiotic policy should be concerned about the remarkably high occurrence of ESBLs. One of the main causes of the current rise in antibiotic resistance to this class is the overuse of third generation cephalosporins to treat gramnegative infections. Extensive use of antibiotics in livestock husbandry generally results in an increase in the amount of extended-spectrum β-lactamase-producing Enterobacteriaceae in animals and consequently in their manure[19]. The discovery of ESBL- and AmpC-producing E. coli in manure from cattle husbandry, as described by Hartmann et al. [20] and Snow et al. [21], confirms this. The issue still exists because, despite widespread recognition in veterinary medicine that ESBL-producing bacteria can cause various infections in dairy cattle, there are still very few studies that look into the prevalence of ESBL- and AmpC-producing bacteria in Nigerian livestock, demonstrating their presence in both sick and healthy cattle, pigs, and poultry.

This supports the idea that there is still a great deal of uncertainty about the possibility of zoonotic transmission from cattle to humans who have intimate contact with these animals. Nonetheless, some research has suggested that farm labourers may have acquired ESBL-producing E. coli or ESBL genes from pigs or poultry[6, 7]. The regular susceptibility test used by clinical laboratories faces several obstacles, one of which is that it typically fails to identify ESBL-positive strains; for this reason, the phenotypic confirmatory test is crucial in the identification of ESBLs. It's still a truth that people can contract zoonotic intestinal bacteria and normal, but resistant, bacterial microflora from animals more frequently through direct contact and animal-to-food interaction.

In addition to colonising people, these resistant bacterial species have the ability to spread resistance genes to other members of the regular bacterial microflora. They may also serve as the primary source of resistance genes and may initiate infections. As a result, the quick spread of resistance genes through mobile gene elements raises the risk and sets the stage for additional therapeutic complications, with a focus on professional groups involved in animal care, such as farmers, veterinary clinics, workers at slaughterhouses, and other individuals involved in the processing of animal foods.

5. CONCLUSION

Enhancing the monitoring of antibiotic usage and antibiotic-resistant microorganisms in farm animals is a critical matter that needs immediate attention. As a result, certain significant steps need to be taken in this direction, particularly with regard to antibiotic sales statistics. Since the majority of them provide data for antimicrobial resistance monitoring programmes, countries should regularly check the levels of antibiotic resistance in farm animals and on retail meat. To create an international surveillance programme for antimicrobial resistance and track developments in antimicrobial resistance in humans and animals over an extended period of time, international governments must work together. The outputs of this activity, both in terms of benefits and risks, must to be taken into account when assessing and managing risks. However, the implementation of biosecurity and hygiene programmes in the cattle breeding industry's intensive sector would have a positive impact on the prevention of the spread of antibiotic resistance. Ultimately, in order to effectively manage antibiotic resistance, a strong approach must be found.

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