INCIDENCE OF ESBLs AND MBLs AMONG ESCHERICHIA COLI AND KLEBSIELLA PNEUMONIAE ISOLATED FROM ABBATOIRS IN ABRAKA AND AGBOR, NIGERIA.

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Abstract

Antibiotic resistance profile, the incidence of extended-spectrum β-lactamases and metallo-β-lactamases among Escherichia coli and K. pneumonia isolates that showed multi-drug resistance of beef samples obtained from abattoirs in Abraka and Agbor, Delta State, Nigeria. A hundred samples of beef specimens were obtained of which fifty samples were obtained from meat samples from different abattoirs located in Abraka and Agbor using a well-labeled sterile swab stick. Thirty-six swab samples comprising 16 samples from freshly butchered meat, 10 from the apron and 10 samples from the meat seller's tables were aseptically collected and bacteriologically analyzed following standard procedure. The bacterial isolates most prevalent were Escherichia coli, Staphylococcus aureus, and Klebsiella pneumonia. The zone of inhibition pattern on bacterial isolates from the different abattoirs showed (9) resistant Klebsiella pneumonia, (2) intermediate, and (3) sensitive isolates. Similarly, Escherichia coli were (3) resistant, (4) intermediate, and (5) sensitive isolates. Twelve isolates that were resistant were selected for the combined disk test. The difference in zones of inhibition of combined disk test on meropenem-resistant organisms from samples shows (2) positive Klebsiellasp and (1) Escherichia coli with values ranging from 10-15mm. A total of 2, E. coli isolates and 6, K. pneumonia isolates were found to be resistant to meropenem with IZDs of ≤ 23mm or ≤ 27mm. Only 6 (10%) isolates of E. coli and 4 (12%) isolates of K. pneumoniae were confirmed to be MBL producers. Conclusively, this study shows that abattoirs are reservoirs for food-borne pathogens that are multidrug-resistant in nature

Keywords: Abattoir, ESBLs, MBLs, MDR, IZD

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Introduction

In many parts of the world, particularly developing countries including Nigeria, the detection of resistant genes and/or multi drug resistant (MDR) enzymes responsible for the negative response of pathogenic bacteria to potent antimicrobial onslaught is still badly distinguished in our society [1]. This is because routine antimicrobial susceptibility studies are ineffective in detecting these MDR organisms.

Gram-negative bacteria represent the most relevant reservoir of resistance to antibiotics in the environment and food-producing animal's habours these Gram-negative bacteria which possess multidrug resistant genes together with genes that provoke the production of extended spectrum β-lactamases (ESBLs) and metallo-β-lactamases (MBLs) possess health risks to the human population [2]. These bacteria are also prevalence in food as a result of poor environmental hygiene of food vendors. Food products of animal origin play prominent role in the transfer of antibiotic resistance [3]. This is because antibiotics are used in the rearing of livestock and poultry birds, and the antibiotic

residues in these animals may cause the emergence of resistant bacteria via selective pressure.

There have been complications in the treatment of bacterial infections, this is as a result of the emergence of multi-drug resistant strains of *Escherichia coli* and *Klebsiella pneumoniae*. *E. coli* showed the highest antibiotic resistance trends among the different types of bacteria in Malaysia between 2013 and 2017 [4]. Metallo-β-lactamases (MBLs) and extended spectrum β-lactamases (ESBLs) are beta-lactamase enzymes produced by pathogenic bacteria and gradually found in Gram-negative organisms, mostly in *E. coli* species. Clinical infections with MBLs and ESBLs-producing isolates are associated with higher morbidity and mortality [5].

However, carbapenem resistance due to Metallo-β-lactamases (MBLs) and extended spectrum β-lactamases (ESBLs) production has been gradually reported among clinical isolates of *E. coli* [6,7]. Among Enterobacteriaceae species, the blaIMP and blaVIM genes have been identified throughout the world. The prevalence rate of carbapenem resistance *E. coli* is higher by acquiring MBL including imipenem (IPM) and Verona integron-encoded Metallo-β-lactamase (VIM) around the world. Metallo-β-lactamases (MBLs) which hydrolyze the carbapenems (imipenem, meropenem, and ertapenem) and render them ineffective for treatment [8]. In addition, ESBLs and MBLs are not susceptible to therapeutic β- lactamase inhibitors like sulbactam, tazobactam, or clavulanic acid (Drawz*et al*., 2014). The emergence and uncontrolled spread of carbapenems in Gram-negative bacteria are under threat [9] but normally carbapenems are used for the treatment of infections caused by β-lactam resistant bacteria including extended-spectrum enzymes producers. MBL and ESBL-producing microbes have a complex epidemiology, and they occur predominantly in members of the Enterobacteriaceae family whose reservoirs are the environment and animals [8]. Therefore, the screening for the presence of these enzymes (ESBLs and MBLs) is a useful epidemiological tool for the containment of possible disease outbreak due to these organisms (*E. coli* and *K. pneumoniae*).
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Materials and Methods

Collection of Samples (Meat Samples)

A total of hundred (100) samples of beef specimen were obtained of which fifty (50) samples were obtained from meat samples from different abattoir located in Abraka and Agbor using sterile swab sticks labeled. After collection, the swab sticks were sealed properly and stored at a temperature of 4°C in the fridge prior to use within 2 hrs. of collection and meat inoculated in test tube containing 9ml of sterile water

Test for Microbial Growth and Isolation of Bacteria

The work bench was swabbed using a disinfectant and Nutrient agar previously prepared according to Manufacturers specification and dispensed aseptically on hundred (100) sterile petri- dishes and were inoculated with meat specimen with previously preserved swap stick containing collected specimen labeled (1 – 100). Inoculated Petri-dishes were inverted and placed in an incubator at a temperature of 37 ºC for 24 hrs, after which Petri-dishes were examined for microbial growth, observations were recorded.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test was performed using disc diffusion described previously by Ekwealor et al. [10]. The following antibiotics were tested: cotrimoxazole (25 µg), ciprofloxacin (16 µg), amoxicillin (30 µg), and ampicillin (32 µg) which concentrations are break point of the respective antimicrobial agents.

The table was swabbed using disinfectant and the media used (Muller Hinton agar) was prepared and sterilized according to directions on the label and dispensed aseptically on sterile Petri - dishes accordingly after which dilutions of antimicrobial agents were prepared using sterile disc inserted into the solution of antimicrobial agent, using a sterilized forcep and placed on the agar plates carefully after being allowed to drip. The pregnated agar plate were then incubated at 37°C for 24hrs and observations were recorded.

Results and Discussion

In this study thirty-six (36) swab samples comprising 16 samples from freshly butchered meat, 10 from the apron and 10 samples from the meat-seller's tables were aseptically collected from the abattoir using sterile swab sticks and these were bacteriologically analyzed for the isolation of *E. coli* and *Klebsiella pneumoniae*.

The bacteria isolates were identified based on biochemical test, morphological and gram stain reaction. A total number of twenty-six (26) bacterial isolates were obtained from both location. The bacterial isolates most prevalent were *Escherichia coli, Staphylococcus aureus and Klebsiella pneumoniae.*

The zone of inhibition of antibiotics pattern on bacterial isolates from abattoirs samples shows in appendix 1. The zone of inhibition of antibiotics pattern on bacterial isolates from abattoirs samples shows (9) resistant *Klebsiella pneumonia*, (2) intermediate and (3) sensitive isolates. Similarly, *Escherichia coli* were (3) resistant (4) intermediate and (5) sensitive isolates. It was observed that isolates are resistant to few antibiotics as presented in Table 2.

Table 2: Antibiotic Profile of Gram-negative Bacterial Isolates in Abattoir

Organisms	Zones of inhibitions (mm)	Resistance (≤ 12)	Intermediate (20-6)	Sensitive(≥ 8)
	Klebsiellaneumonia			
HK1	23	+	-	-
Hk2	33	+	-	-
HK3	27	+	-	-
HK4	28	+	-	-
HK5	30	-	+	-
HK6	26	-	-	+
HK7	25	+	-	-
HK8	0	+	-	-
HK 9	20	-	-	+
HK 10	0	-	-	+
HK 11	33	+	-	-
HK 12	20	-	+	-
HK 13	26	+	-	
HK 14	24	+	-	
	Escherichia coli			
HE1	21	-	-	+
HE 2	10	+	-	-
HE 3	20	-		+
HE 4	26	-	+	-
HE 5	23	-	+	-
HE 6	20	-	-	+
HE 7	20	-	-	+
HE 8	5	+	-	-
HE 9	15	-	+	-
HE 10	10	-	-	+
HE 11	5	-	+	-
HE 12	14	+	-	-

Twelve isolates that was resistant were selected for combined disk test.

The difference in zones of inhibition of combined disk test (CDT) on meropenem resistant organisms from samples shows (2) positive *Klebsiella*spand (1) *Escherichia coli* with the values ranging from 10-15. As presented in Table 3.

Table 3: Zone of Inhibition of Combined Disk Test (CDT) on Resistance Organisms from Samples

Organisms	*MEM*	*MEM+EDTA*	*Positive or negative*
E1	-	-	-
E2	7	18	(11^{+}) positive
E3	-	-	-
K1	16	11	≥
K2	-	-	-
K3	-	-	-
K1	11	21	(10^{+}) positive
K2	10	11	-

K7	8	23	(15^{+}) positive
K3	20	16	-
K4	28	26	-
K10	13	17	-

K2-*Klebsiella*sp

Twelve isolates that was resistant were selected for double disk synergy test. The difference in zones of inhibition of double disk synergy test on meropenem resistant organisms from samples shows (3) positive *Klebsiella*sp and (1) *Escherichia coli* with the values ranging from 10-15 as presented in Table 4.

Table 4: Zone of inhibition of Double Disk Synergy Test on Resistance Organisms from Samples (detection of metallo-betalactamases)

Organisms	***MEM+EDTA***	***MEM***	***EDTA***	***Positive or negative***
E1	14	20	15	Positive
E2	-	-	-	-
E3	-	-	-	-
K1	-	-	-	-
K2		-	-	-
K3	-	-	-	-
K1	18	23	22	Positive
K2	17	25	17	Positive
K7	-	-	-	-
K3	-	-	-	-
K4	-	20	3	-
K10	17	25	12	Positive

A total of 2, *E. coli* isolates and 6, *K. pneumoniae* isolates were found to be resistant to meropenem with IZDs of ≤ 23mm or ≤ 27mm. But only 6 (10%) isolates of *E. coli* and 4 (12%) isolates of *K. pneumoniae* were confirmed to be MBL-producers (Table 3.5).

Table 5: Results of Metallo-β-lactamase (MBL) detection (%)

Bacterial Isolates	**MBL screen positives**	**MBL nonproducing**	**MBL producing**
K. pneumoniae	20% (12)	3.2% (2)	10% (6)
E. coli	15.8% (6)	3.0% (1)	11.8% (4)

Food-producing animals serve as reservoirs and or routes for the spread of antibiotic resistant bacteria in the community through the food chain [11,12]. Thirty six (36) abattoir samples was investigated for the presence of gram negative bacteria with *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* being more prevalent. Out of the (36) samples, twenty six gram negative organisms were isolated. This could also have been as a result of the exposure of the meat to the atmosphere, water used for preparation or microbes from handlers. This result is in agreement with the studies conducted by Enemor*et al*., [13] who reported that the presence of microorganisms in the meat samples indicated high contamination in the meat which is unbearable for human consumption. However, this is in agreement with the report of Amusa*et al*. [14] and Akbar and Anal [15]. The presence of coliforms could be traced to poor sanitary practices of food handlers and is an indication of faecal contamination [16](Ameko*et al*., 2012).

The biochemical test identification and utilization of sugars carried out to identified the isolates as revealed in Table 1, shows the presence of *Escherichia coli* and*Klebsiella pneumoniae* among others organisms. In this study, presence of beta lactamase production in *E. coli* and *K. pneumoniae* isolates were screened for, from raw/ freshly slaughtered animals, apron and Meat seller's tables in a local abattoir. The results revealed high incidence of *K. pneumoniae* isolates than *E. coli*. The incidence recoded in this study concurs with the report of Addis and Sisay, to be the major cause of food borne infection [17]. *K. pneumoniae* though not a commonly known bacteria found in animal intestine, has been reported as an opportunistic

pathogen of humans, animals, and a common contaminant of retail meats [18].This also supported the finding of [19].

Meropenem among the beta-lactams are the most effective against gram negative bacteria presenting a broad spectrum of antibiotics activity. The zone of inhibition of antibiotics pattern on bacterial isolates from abattoirs samples shows that *Klebsiella pneumoniae* were more frequently resistant than *Escherichia coli* while others were sensitive to Meropenem. Furthermore, the high colonization rate could be attributed to cross contamination of meats in abattoirs particularly during slaughtering. The processes of slaughtering are potential risk factors that may exacerbate the transmission rate of beta-lactamase producing *E. coli* resistant strains [20]. The result of antimicrobial susceptibility testing revealed an interesting pattern with resistance rates observed in the majority of antimicrobial agents tested especially amongst the beta-lactam and macrolides groups. Most of the isolates obtained were multi-drug resistant. Since majority of the resistance were against β-lactam antibiotics the resistance pattern might be by the inactivation of β-lactam ring by the β-lactamases as most of these enzymes are constitutive in Gram-negative organisms [21].

The difference in zones of inhibition of combined disk test (CDT) on meropenem resistant isolates shows (2) *Klebsiella pneumoniae* positive and (1) *Escherichia coli* with the range of 10-15. The resistance in the organisms could be due to the fact that antibiotics was unable to penetrate organisms. This support the finding of Yong [22]. However, high meropenem prevalence not only complicates antibiotic therapy but also interfere with empirical therapy resulting in increased morbidity and mortality. The prevalence and degree of occurrence of one or two of these organisms over others are dependent on the environment [23].

The difference in zones of inhibition of double disk synergy test on meropenem resistant organisms shows that (3) positive *Klebsiella*spand (2) *Escherichia coli* with the values ranging from 10-15. The production of metallo-beta lactamase (double disk synergy) by these organisms may probably be due to its inhibitory ability against the antibiotics. The percentage production of metallo-beta lactamase by these organisms could be attributed to the potency of the organisms to survive the antibiotics or due to the mechanism of action. This result is in agreement with of the work of Antonela *et al*., [24] who reported on binding force of bacteria isolates. The high levels of antibiotics production and multi-drug resistance of the isolates are indications of an increase in the resistance menace reported by Malini and Aditi, [25]. However, it could also be due to its characteristics to exhibit resistance. This is similar to the work of 'Ejikeugwu*et al*., [24]. This ESBL result differed from what Ismail and Haydar whose report in Brazil where 80% of *E. coli* and 3.6% *K. pneumoniae* recovered from foods of animal origin were ESBL. These findings reflected a high incidence of MBL-producing *E. coli* and *K. pneumoniae* from local abattoir with a great risk and possibility of other forms of antibiotic resistance. This result however differed from the work of Ejikeugwu with 28.6% MBL producing *E. coli* recorded from a slaughter house in a neighboring state in Nigeria [26].

Conclusion

Conclusively, this study shows that abattoir can be reservoir for food borne pathogens that are multidrug resistant in nature. And the high incidence of these organisms in our study coupled with their high antibiotic resistance profile reflects poor handling of the meat products and undue use of antibiotics in the production of these animals. (Efficient and periodic surveillance programmes should be encouraged to monitor ever shifting prevalence and antibiogram patterns)

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