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RESEARCH ARTICLE

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OCCURRENCE OF HIGH FLUOROQUINOLONE RESISTANCE AMONG KLEBSIELLA PNEUMONIA FROM APPARENTLY HEALTHY CATTLE AND SWINE IN ADO - EKITI, NIGERIA.

ABSTRACT

Fluoroquinolones are new generations of antibiotics and are increasingly used in human and veterinary medicine. We tested isolates of 63 and 50 isolates of Klebsiella pneuminiae from cattle and swine respectively from different locations in Ado Ekiti, Nigeria for the susceptibility to fluoroquinolones. Using the agar disk diffusion test, susceptibilities to the following fluoroquinolones were tested: pefloxacin (5µg), norfloxacin (5µg), levofloxacin (5µg), ciprofloxacin (5µg) and enrofloxacin (5µg). Klebsiella pneumonia isolates cultured on Mueller Hinton agar plates were incubated at $37^{O}C$ for 24 hours. All plates were examined for zones of inhibition which were measured and appropriately interpreted using standard baseline as specified by the Clinical Laboratory Science Institute. The isolates recovered from cattle showed the highest resistance to levofloxacin 46/63(73.0%) while those isolates from swine showed highest resistance to norfloxacin 44/50 (88.0%) and pefloxacin 44/50 (88.0%). The bacteria showed high level of multiple fluoroquinolone resistance; 13 different fluoroquinolone resistance phenotypes were observed among cattle while 10 phenotypes were observed among swine. These findings buttress the need for proper antibiotic stewardship regarding the use of the fluoroquinolones in animals.

Keywords: Klebsiella pneumonia, antibiotics, resistance, cattle, swine

1.0 INTRODUCTION

Klebsiella pneumonia is a gram negative and enteric bacterium that is widespread in different environments and carried by humans, insects and animals (Bouza and Cercenado, 2002; Dillon et al., 2002). Despite the preponderance of the Klebsiella spp. as normal flora in different environments including the intestinal flora of humans and animals, they have a rich history as causative agents of different infections and are also frequently implicated in many nosocomial infections (Yu et al., 2007; Marra et al., 2006; Tsai et al., 2010). The most prominent species among the genus Klebsiella are Klebsiella pneumonia and Klebsiella oxytoca and which are frequently found in humans and animals (Bleich et al., 2008; MacArthur et al., 2008).

Although antibiotics have an enormous importance in human medicine due to their use in treatment of infections in humans primarily to reduce morbidity and mortality that may be occasioned by such infections, they are also frequently deployed at sub-therapeutic levels in animals in food animals to promote growth, prevent and controls infections (Schroeder et al., 2002; Felding et al., 2010; Landers et al., 2012). The antibiotics that are commonly licensed for use in cattle and swine include tetracyclines, pennicillins, bacitracin, etc and their various therapeutic and nutritional benefits justify their uses (USDA).

The fluoroquinolones are among the latest generations of broad spectrum synthetic antibiotics that have been used extensively for different therapeutic purposes in humans and are currently finding an increased use in animals (Collignon et al., 2005; Karlsson et al., 2010). There is a huge body of scientific evidence that the extensive use of antibiotics, including the fluoroquinolones, are intricately linked to the increasing incidence of antibiotic resistance among animals with implications for public health and food safety. This present study therefore seeks to determine the presence of fluoroquinolone resistance among Klebsiella pneumoniae among apparently healthy cattle and swine at different locations in Ado Ekiti, Ekiti State, Nigeria.

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1.1 MATERIALS AND METHODS

1.1.1 Sample collection

Faecal swabs were collected from apparently healthy cattle that were being prepared for slaughter at the State abattoir in Ado Ekiti between February and May, 2013. The swabs were also collected from the apparently healthy swine at three different locations within the state; including Ado Ekiti. All samples were taken to the Microbiology Laboratory, Ekiti State University and processed within 24 hours.

1.1.2 Isolation and biochemical identification of K. pneumonia

All samples were initially pre-enriched by inoculating into test tubes containing tryptone soy broth and incubated at 37^{O}Cfor 6 hours. They were subsequently inoculated onto MacConkey agar plates and incubated at 37^{O}C for 18-24 hours. After incubation, all plates were examined for suspect and distinct colonies that were characteristic of the Klebsiella spp. All suspect colonies were further sub-cultured onto sterile MacConkey agar plates and incubated as appropriate. Such colonies were subjected to extensive biochemical tests which include indole test, methyl red test, citrate utilization test and urease test. Representative isolates that were confirmed as K. pneumonia were stored into slants and kept at 4^{O}C for further studies.

1.1.3 Antibiotic susceptibility tests

All the isolates of Klebsiella pneumonia were examined for susceptibility to the selected fluoroquinolones using the agar disk diffusion method according to the CLSI, (2010). The antibiotics that were tested included pefloxacin (5µg), norfloxacin (5µg), levofloxacin (5µg), ciprofloxacin (5µg) and enrofloxacin (5µg). Isolates were inoculated onto Mueller Hinton agar plates and incubated at 37^{O}C for 24 hours. The innocula for all susceptibility tests were standardized using the 0.5 McFarland standard. All plates were examined for zones of inhibition and such zones were measured in millimeters. Susceptibilities were interpreted using standard interpretative criteria.

1.2 RESULTS AND DISCUSSION

One hundred and thirteen isolates were recovered from cattle and swine of which sixty three isolates of K. pneumonia were from cattle while fifty isolates from swine. All isolates from cattle and swine at various locations were subjected to susceptibility tests to reveal their sensitivity to fluoroquinolones. Overall results revealed that the Klebsiella pneumonia among apparently healthy cattle showed various levels of resistance to fluoroquinolones. Resistance to levofloxacin was highest among cattle 46/63 (73.0%) while resistance to pefloxacine was the least 5/63 (8.0%) (Table 1). The K. pneumoniae isolates from swine showed the highest resistance to levofloxacin and pefloxacin with 44 (88%) each of the isolates showing resistance each to the antibiotics (Table 1). Fifty five of the isolates from cattle showed multiple resistance patterns with the most prominent resistance observed to be enr/nor/lev/cip/pef with 14/63 (22.2%) isolates among cattle showing this pattern (Table 2). Eleven different multiple fluoroquinolone resistance patterns were also observed among the isolates from swine with the most prominent resistance patterns appeared to be enr/nor/lev/cip/pef observed in 19 isolates of K. pneumonia (Table 2).

S/N	Antibiotics	Cattle (n=63)	Swine (n=50)
1	Enrofloxacin	40 (63.5%)	31 (62.0%)
2	Norfloxacin	31 (49.2%)	44 (88.0 %)
3	Levofloxacin	46 (73.0%)	39(10.0%)
4	Ciprofloxacin	34 (54%)	27 (7.0%)
5	Pefloxacin	5 (8.0%)	44 (88.0%)

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Table 1: Overall resistance to fluoroquinolones among K. pneumonia from cattle and swine to single antibiotics.

S/N	ANTIBIOTIC RESISTANCE PHENOTYPE	CATTLE (n=63)	SWINE (n=50)
1	Cip-Pef	1	-
2	Lev-Pef	2	-
3	Enr-Pef	2	-
4	Lev-Pef	-	2
5	Nor-Lev	-	1
6	Nor-Pef	1	-
7	Lev-Cip-Pef	5	4
8	Enr-Lev-Pef	4	-
9	Enr-Nor-Pef	3	-
10	Nor-Lev-Pef	2	2
11	Nor-Lev-Enr	-	1
12	Nor-Lev-Cip	-	1
13	Enr-Cip-Pef	3	-
14	Nor-Lev-Cip-Pef	4	6
15	Lev-Cip-Enr-Pef	-	1
16	Nor-Lev-Enr-Pef	7	1
17	Enr-Nor-Cip-Pef	1	-
18	Nor-Lev-Cip-Enr-Pef	14	21

Table 2: Multiple fluoroquinolone resistance patterns to among K. pneumoniae from cattle and swine.

This study determined the incidence of resistance to fluoroquinolones among isolates of Klebsiella pneumonia that were recovered from apparently healthy cattle and swine at selected locations in Ado Ekiti, Nigeria. The resistance among the isolates from cattle to levlfloxacin was highest while those isolates that were recovered from swine showed the highest level of resistance against norfloxacin and pefloxacin. Overall, this present study has confirmed that resistance against broad spectrum fluoroquinolones among Klebsiella pneumonia among cattle and swine is high and many isolates showed unprecedented levels of multiple resistance to fluoroquinolones. This important finding has serious implications and a major cause for serious concern.

Studies have correspondingly confirmed increasing incidence of fluoroquinolone resistance among bacteria from different origins, which include hospitals, food and companion animals. Nordmann and Poirel (2005) and Hooper, (2001) reported the emergence of resistance to fluoroquinolones among Enterobacteriacaea and such resistance is increasing globally. Mustak et al., 2012 observed that enteric bacteria, most especially Escherichia coli strains from animals also show resistance to fluoroquinolones. Ma et al., (2009) reported a high prevalence of quinolone resistance among Klebsiella pneumonia that were recovered from food and companion animals. The European Medicine Agency (2006) provided further insight into the increasing problem of resistance to fluoroquinolones among bacteria with food and companion animals as the origin. Ajayi et al., 2012 reported the presence of fluoroquinolone resistance in commensal E. coli recovered from among apparently healthy cattle. Yang et al., (2004) characterized multiple resistant enteric bacteria, particularly E. coli from chicken and swine. Emergence of fluoroquinolone resistant bacteria from swine was also reported by Li et al., (2002).the results obtained from our present study further buttresses the problem of resistance to antibiotics, especially the fluoroquinolones.

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The prevalence of fluoroquinolone resistance among K. pneumonia that were recovered from cattle and swine in this present study was notably high. More serious concern is the high incidence of multiple resistance to fluoroquinolone as many isolates showed resistance to all the fluoroquinolones that were tested. This is consistent with similar findings that have also confirmed the increasing incidence of multiple resistance to fluoroquinolones among bacteria from different animal origins (Lin and Davies, 2007; Ajayi et al., 2011). The predominance of highly fluoroquinolone resistant commensal K. pneuminiae isolates among apparently healthy cattle and swine within the study locations suggests the use of such antibiotics. The use of fluoroquinolones in domestic and food animals is generally considered as a major factor for the emergence of resistance to fluoroquinolones among enteric bacteria (Lin and Davies, 2011). The swine breeding industry, in particular, is heavily reliant on the use of antibiotics, fluoroquinolones and cephalosporins, and such heavy dosing of animals with antibiotics could drive the emergence of fluoroquinolone resistant bacteria in swine (Chee-Sanford et al., 2001; Mackic et al., 2006). Growing scientific evidence have consistently linked the upsurge in the prevalence of fluoroquinolone resistant infections to the use of the fluoroquinolones for different purposes in animals (Gupta et al., 2004; Iovin and Blaser, 2004). Antibiotic resistant bacteria frequently emerge from animals and are subsequently transmitted to humans through contaminated food chains and water (Angulo et al., 2000).

Following the discovery of fluoroquinolones, they have emerged as the latest drugs of choice for the treatment of a wide variety of infections in humans (Fu et al., 2013; Somasundaram and Manirannan, 2013) and animals (Colignon, 2005). In some cases, the fluoroquinolones are used as antibiotics of last resort whenever other options of antibiotics have failed. Levofloxacin, for instance, is considered one of the newest fluoroquinolones and the high incidence of resistance against this antibiotic among isolates of K. pneumonia in cattle and swine in this present study may have serious implications for human health, animal health and food safety.

1.3 CONCLUSION

The specific mechanisms of resistance to the fluoroquinolones and the detailed survey of uses of fluoroquinolones in the areas of this study are crucial aspects for further studies. This study calls for appropriate stewardship for the use of fluoroquinolones in order to preserve their efficacy.

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