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Occurrence of Multi-drug Resistant Gram Negative Bacteria from Poultry and Poultry Products sold in Abakaliki Metropolis, Nigeria

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ABSTRACT

This study determined the frequency and antibiogram of *Salmonella* species, *Escherichia coli* and *Pseudomonas aeruginosa* isolated from environmental samples. A total of 79 environmental samples of poultry origin including meat (n= 21), egg content (n= 16), drinking water (n= 7), feed (n= 7), egg shell (n= 16) and droppings (n= 12) were used for this study and bacteriologically analyzed by classical cultural methods. All isolated bacteria were identified using standard microbiological identification technique. The antimicrobial susceptibility profile of the isolates was determined by the Kirby-Bauer disk diffusion technique. Overall, 70 bacteria isolates comprising of *Salmonella* species (n=58), *E. coli* (n=10) and *Pseudomonas* species (n=2) were isolated from the samples of poultry origin; all of them were characterized as multidrug resistant. *E. coli* and *Salmonella* isolates were highly resistant to tetracycline, nalidixic acid, sulphamethoxazole-trimethoprim, nitrofurantoin and cefotaxime, while *P. aeruginosa* isolates were highly resistant to β-lactams used, tetracycline, tobramycin, nitrofurantoin and sulphamethoxazole-trimethoprim. On contrary, ofloxacin, imipenem and ertapenem were highly effective against examined bacterial pathogens. This study showed that poultry products harbor potential multidrug resistant bacteria which could cause diseases in humans, so, prompt and accurate detection of these pathogens is vital to the containment of their emergence and spread.

Key words: *Salmonella, Escherichia coli, Pseudomonas aeruginosa,* Poultry, Antibiotic Resistance and Nigeria.
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INTRODUCTION

Bacteria resistance to some commonly available antimicrobial agents continues to remain a significant public health concern worldwide. The development and spread of antimicrobial resistant bacteria has increased dramatically in the community (Hart, 1998; Wilson, 2004; Okoli et al., 2006; Oyeleke, 2009; Duru et al., 2013) and the entire population may be at risk of acquiring drug resistant bugs via the consumption of animal products or through direct contact. Feeds used for rearing of poultry served as fomites in the transference of antimicrobial resistant organisms to the community (Kariuki et al., 2002). Additionally, improperly handled eggs and egg products could be a source of food borne diseases, such as *Salmonellosis* (Heriskstad et al., 2002). The wide spread and improper application of antimicrobial agents in the production livestock and poultry birds have been linked to some infections in human population; and direct contact with infected animals may also serve as a source of infections with drug resistant organisms (Molbak et al., 1999; Tauxe et al., 2004). Several human practices such as overcrowding, unhygienic farming activities, lack of adequate biosecurity measures and movement of birds and equipment from one farm to the other are some notable risk factors that allow drug resistant microbes to

emerge and spread through poultry products (Davies et al., 1997).

Several factors contribute to the antibiotic resistance problem that we now face in both the community and in the hospital environment; and most of these factors have to do with both human behavior and activities while the other factors are contributed by the microbes themselves. The first among these factors that contribute to the development of antibiotic resistance is “natural selection” which allows microbes to over time adapt in ways which increases their ability to survive in a changing environment such as that provided by antimicrobial agents (STRAMA, 2007). It is also noteworthy that the human application of toxic agents including antibiotics on massive scale (such as is applicable in the rearing of poultry birds and food-producing animals) activates the genetic elements of microbes to promote the survival of the microbial population in any environment. Since the excessive use of antibiotics in livestock and animal feeds contribute to the emergence and spread of antimicrobial resistant pathogens in the community, this study presumptively evaluated the occurrence of antibiotic resistant Gram negative bacteria from poultry products in Abakaliki metropolis, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Collection and Processing of Samples

A total of 79 environmental samples of poultry origin including meat (n= 21), egg content (n= 16), drinking water (n= 7), feed (n= 7), egg shell (n= 16) and droppings (n= 12) were used for this study. The samples were aseptically collected into clean zip lock bags using a clean spatula and swab sticks; and each of the sample containers were labeled and transported to the microbiology laboratory of Ebonyi State University, Abakaliki for further microbiological analysis.

Bacteriological Analysis

One gram each of the samples was inoculated into nutrient broth (Oxoid, UK) tubes and the tubes were incubated at 35ºC for 18 to 24 h. Tubes showing turbidity after incubation were bacteriologically cultured on brilliant green agar, xylose-lysine deoxycholate (XLD) agar, Salmonella-Shigella agar (SSA), and MacConkey agar (MAC) for the isolation of bacteria, and the culture plates were incubated at 35ºC for 18 to h (Cheesbrough, 2000). All culture media were procured from Oxoid limited (Oxoid, UK). Suspect bacterial colonies on the culture media plates were aseptically subcultured onto freshly prepared SSA, MAC, XLD and brilliant green agar for the isolation of pure cultures. And the isolated organisms were purified on nutrient agar plates for further bacteriological studies.

Identification of Bacteria

Suspect bacterial isolates on the respective culture media plates were identified using standard microbiological identification techniques including motility test, indole, triple sugar Iron test, H_2S production test, sugar fermentation test, citrate utilization test, Voges-Proskauer test, and Methyl-red test (Cheesbrough, 2000).

Antibiogram

Antimicrobial susceptibility studies were carried out as per the guideline of the Clinical Laboratory Standard Institute (CLSI) using the Kirby-Bauer disk diffusion method. Single antibiotic disks including ceftriaxone (CRO, 30 µg), penicillin G (P, 10 µg), gentamicin (CN, 30 µg), amoxycillin (AML, 25 µg), nitrofurantoin (F, 300 µg), tetracycline (TE, 10 µg), amoxycillin/clavulanic acid (AMC, 30 µg), sulphamethoxazole-trimethoprim (SXT, 25 µg), ofloxacin (OFX, 5 µg), cefotaxime (CTX, 30 µg), tobramycin (TOB, 30 µg), ceftazidime (CAZ, 30 µg), imipenem (IPM, 10 µg), ertapenem (ETP, 10 µg) and nalidixic acid (NA, 30 µg). All the antibiotic disks were procured from Oxoid limited (Oxoid, UK) were used for the antibiogram studies as was previously described (Ejikeugwu et al., 2012; Duru et al., 2013). Briefly, the test isolate (adjusted to 0.5 McFarland turbidity standards) were aseptically swabbed on the surface of Mueller-Hinton (MH) agar plates, and the antibiotic disks were aseptically inserted into the MH agar plates using sterile forceps. Inoculated plates were incubated at 35ºC for 18 to 24 h, and the inhibition zone diameter (IZD) were recorded and reported as per the CLSI standards based on the organisms susceptibility and resistance profile.

RESULTS

A total of 79 poultry samples including samples from poultry bird (chicken) meat (n=21), poultry eggs (n=16), poultry birds drinking water (n=7), poultry feed (n=7), egg shell (n=16) and poultry droppings/feacal samples (n=7) were bacteriologically examined (Table 1). Among these poultry samples examined, (Table 2). Table 2 show the overall percentage prevalence of the bacteria isolated from the poultry samples. The most isolated bacteria from the poultry samples were *Salmonella* species and *E. coli* while *P. aeruginosa* was the least isolated bacterium. A total of 70 bacteria isolates including *Salmonella* species, *E. coli* and *P. aeruginosa* were recovered from the various poultry samples used in this study. Among the bacteria isolated, 58 (82.86%) isolates were considered positive for *Salmonella* species. The isolated *Salmonella* species were recovered from all the poultry samples used

Table 1. Distribution of bacteria isolated from poultry farms and outlets.

Sample Collection Source (n)	Isolated Organism	No of Organism Isolated
Feeds (7)	*Salmonella* species	4
Feacal samples (12)	*Salmonella* species	9
Drinking water (7)	*Salmonella* species	7
Egg shell (16)	*Salmonella* species	8
	Escherichia coli	14
	Pseudomonas aeruginosa	1
Egg content (16)	*Salmonella* species	10
	Escherichia coli	15
	Pseudomonas aeruginosa	1
Poultry meat (21)	*Salmonella* species	20
	Escherichia coli	21
n=number of sample		

Table 2. Percentage distribution of the bacterial pathogens.

Organism	No (%)
Salmonella species	58 (82.86)
Escherichia coli	10 (14.29)
Pseudomonas aeruginosa	2 (2.85)
Total	70

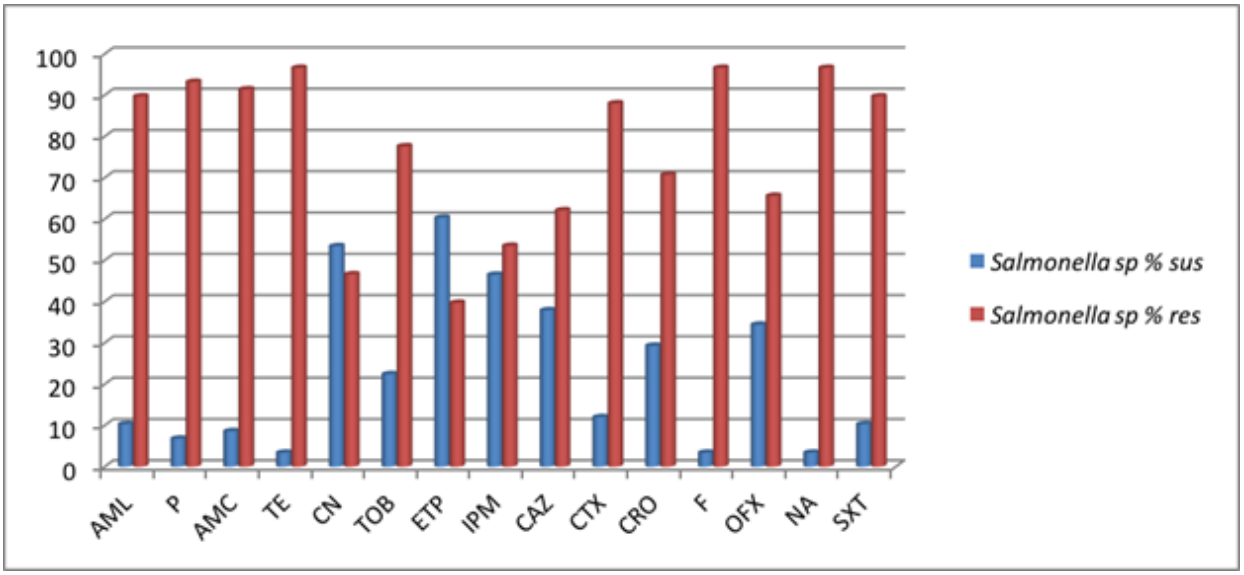

Figure 1. Antibiogram of *Salmonella* species isolated from poultry samples and its products. **KEYS:** AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin, ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime, CTX = Cefotaxime, CRO = Ceftriaxone, F = Nitrofurantoin, OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole /Trimethoprim.

in this study. A total of 10 (14.29%) *E. coli* isolates and 2 (2.85%) *P. aeruginosa* isolates were recovered from the poultry samples (Table 2).The *E.coli* isolates was isolated from egg shell samples, egg content samples and poultry meat samples as shown in Table 1. *E. coli* isolates was not recovered from the poultry feed samples, poultry drinking water and poultry feacal samples. *P. aeruginosa* isolates was recovered from only egg shell samples and egg content samples. Antimicrobial susceptibility testing was performed on all the isolated *Salmonella* species, *E. coli* and *P.seudomonas aeruginosa* isolates using 15 single antibiotic disks from various antibiotic classes.

The antimicrobial susceptibility profile of the isolated *Salmonella* species, *E. coli,* and *P. aeruginosa* isolates is shown in Figures 1, 2, and 3. The result of the antimicrobial susceptibility testing shows that the

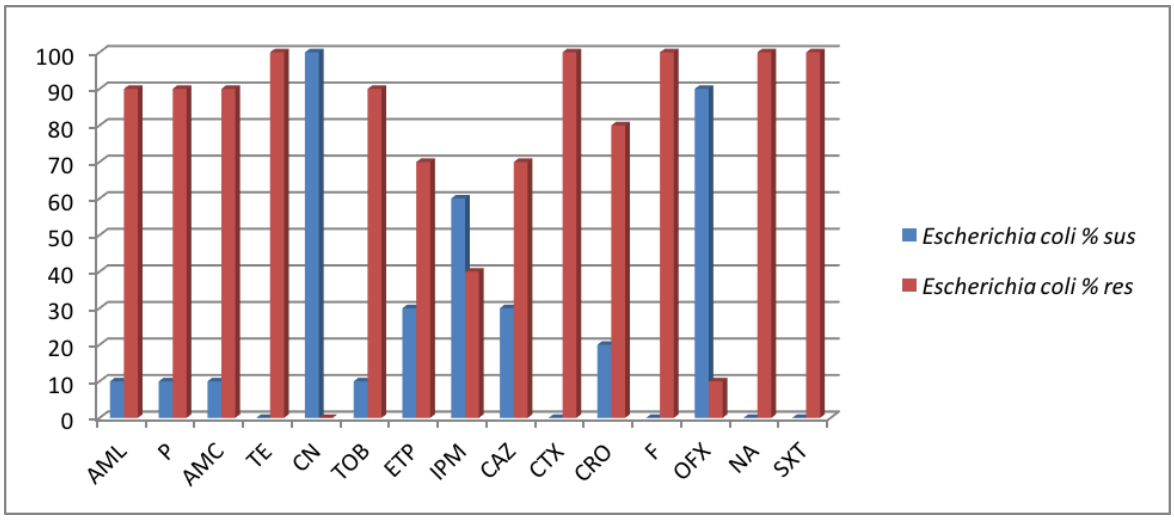

Figure 2. Antibiogram of *E. coli* isolated from poultry samples and its products.

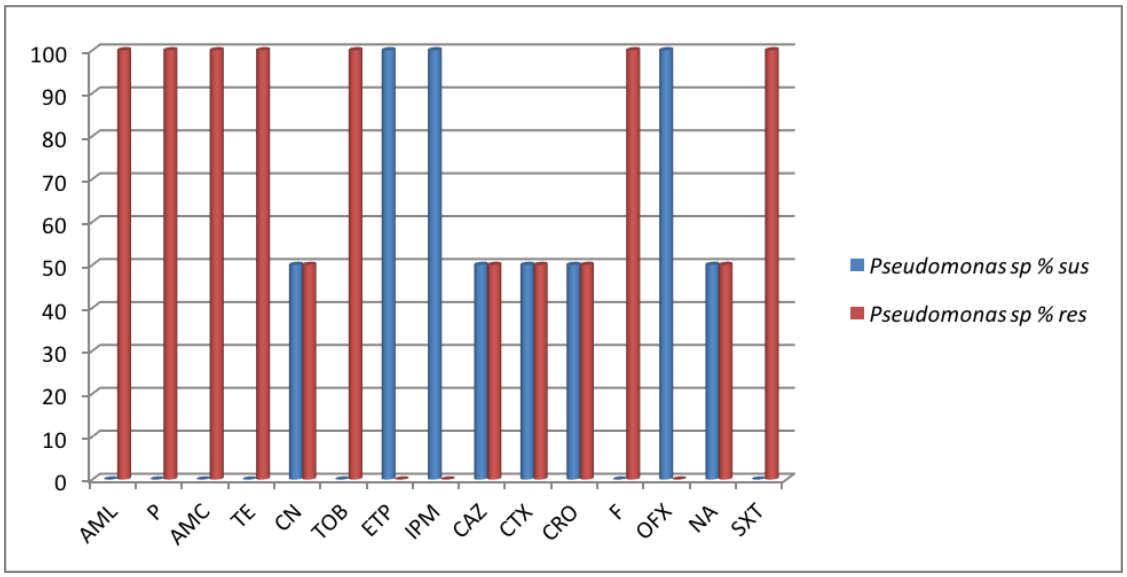

Figure 3. Antibiogram of *Pseudomonas* species isolated from poultry samples and its products. **KEYS:** AML = Amoxycillin, P = Penicillin, AMC = Amoxycillin/clavulanic acid, TE = Tetracycline, CN = Gentamicin, TOB = Tobramycin , ETP = Ertapenem, IPM = Imipenem, CAZ = Ceftazidime , CTX = Cefotaxime , CRO = Ceftriaxone, F = Nitrofurantoin , OFX = Ofloxacin, NA = Nalidixic acid, SXT = Sulphamethoxazole /Trimethoprim.

Salmonella species were most susceptible to ertapenem, imipenem and gentamicin. However, the isolated *Salmonella* species were highly resistant to nalidixic acid, tetracycline, sulphamethoxazole-trimethoprim, cefotaxime, amoxicillin, penicillin, nitrofurantoin and amoxicillin-clavulanic acid. They were also resistant to tobramycin, ceftazidime, ofloxacin and ceftriaxone (Figure 1). Figure 2 shows the susceptibility profile of the isolated *E. coli* isolates to all the tested antibiotics. The *E. coli* isolates also showed varying rates of resistance and susceptibility to the tested antibiotics. All the *E. coli* isolates recovered from the poultry sample was completely resistant to nalidixic acid, sulphamethoxazole-trimethoprim, cefotaxime, nitrofurantoin and tetracycline (Figure 2). There was a 100% resistance profile of the *E. coli* isolates to TE, CTX, F, NA and SXT. However, the *E. coli* isolates were highly susceptible to gentamicin, ofloxacin and imipenem. Gentamicin was very effective against the *E. coli* isolates as was observed in the *Salmonella* species. Least susceptibility of the *E. coli* isolates was also observed against amoxicillin, penicillin, amoxycillin-clavulanic acid, tobramycin, ertapenem, ceftazidime and ceftriaxone (Figure 2). Figure 3 shows the susceptibility profile of the *P. seudomonas*

aeruginosa isolates to the tested antibiotics. The *P. aeruginosa* isolates were the most resistant bacteria pathogens to the tested antibiotics; and they were completely resistant to amoxicillin, penicillin, amoxycillin-clavulanic acid, tetracycline, tobramycin, nitrofurantoin, and sulphamethoxazole-trimethoprim. However, they were completely susceptible to ertapenem, imipenem and ofloxacin. Gentamicin, ceftazidime, cefotaxime, ceftriaxone and nalidixic acid showed appreciable antimicrobial activity against the *P. aeruginosa* isolates irrespective of their notable resistant nature.

DISCUSSION

The use of antibiotics in animal-food production contributes to the continued emergence and dissemination of drug resistant bacteria pathogens in the community especially in poultry farms. *Salmonella* species, *E. coli* and *P. aeruginosa* are common bacterial pathogens implicated in most foodborne infections; and these organisms which are known as the leading cause of gastroenteritis in humans can be transmitted through food especially those of poultry origin as examined in this study. This study was conducted to determine the prevalence and antimicrobial susceptibility profile of some bacterial pathogens commonly encountered in some poultry products and samples of poultry origin. A total of 79 environmental samples of poultry origin including poultry bird meat, poultry eggs, drinking water of poultry birds, poultry feed, egg shell and poultry droppings/feacal samples were bacteriologically examined in this study for the isolation of common bacterial pathogens associated with poultry samples. It was observed in our study that a total of 70 bacterial isolates comprising of *Salmonella* species (n=58), *E. coli* (n=10) and *Pseudomonas* species (n=2) were bacteriologically isolated from the poultry samples examined in this study. *Salmonella* species and *E. coli* appeared to be the most prevalent bacterial species isolated from the poultry samples whereas *Pseudomonas* species were the least bacteria isolated.

It has been previously reported that higher prevalence of commensal bacterial flora contributes to the general increase and dissemination of bacterial pathogens worldwide; and that these organisms can aid in the transmission of antibiotic resistant traits (Chikwendu et al., 2008). The bacterial species isolated in this study could pose a potential public health risk since these organisms are potential causative agents of some infectious disease of man. It has also been reported that the presence of these organisms in the community especially in food and water could reflect the hygienic condition of the particular environment; and that they could also cause both waterborne and foodborne infections (Ekhaise et al., 2008; Okoli et al., 2006; Duru et al., 2013; Whyte et al., 2003; Johnson et al., 2007). The result of the antibiogram of *Salmonella* species to the tested antibiotics shows that the *Salmonella* species were most susceptible to ertapenem, imipenem and gentamicin. However, they were highly resistant to nalidixic acid, tetracycline, sulphamethoxazole-trimethoprim, cefotaxime, amoxicillin, penicillin, nitrofurantoin and amoxicillin-clavulanic acid–some of which are commonly used for the treatment of infections caused by *Salmonella*. The *Salmonella* species were also resistant to tobramycin, ceftazidime, ofloxacin and ceftriaxone. Overall, the *Salmonella* species were resistant to 13 (86.7%) out of the 15 antibiotics used in this study. A similar antibiotic resistance profile of *Salmonella* species isolated from chickens (as obtainable in this study) has also been reported elsewhere where *Salmonella* was found to be highly resistant to tetracycline and gentamicin (Wilson, 2004). Multidrug resistant *Salmonella typhimurium* strains have also been reported in food animals (Wilson, 2004; STRAMA, 2007). The percentage resistance of *Salmonella* species to the tested antibiotics in this study was however lesser than that reported by Abdellah et al. (2009) who reported 75.43% resistance of *Salmonella* isolates some commonly used antibiotics.

The *Salmonella* species isolated in this study showed 100% resistance to tetracycline, a commonly used growth-promoting agent in poultry farms. A previous study has shown that tetracycline is usually utilized as antibiotic growth supporters in the production and rearing of animals (Jones and Ricke, 2003); and this practice could contribute to the drug resistant nature of the organism. The *E. coli* isolates also showed varying rates of resistance and susceptibility to the tested antibiotics. All the *E. coli* isolates recovered from the poultry sample was completely resistant to nalidixic acid, sulphamethoxazole-trimethoprim, cefotaxime, nitrofurantoin and tetracycline; and this was also followed by a 100% resistance profile of the *E. coli* isolates to tetracycline, cefotaxime, nitrofurantoin, nalidixic acid and sulphamethoxazole-trimethoprim. However, the *E. coli* isolates were highly susceptible to gentamicin, ofloxacin and imipenem. Gentamicin was very effective against the *E. coli* isolates as was observed in the *Salmonella* species. Least susceptibility of the *E. coli* isolates was also observed against amoxicillin, penicillin, amoxycillin-clavulanic acid, tobramycin, ertapenem, ceftazidime and ceftriaxone. *E coli* isolated from this study were resistant to 12 (80%) antibiotics out of the 15 antibiotics used in this study. It is notable that many pathogenic strains of *E. coli* have a relatively large potential for developing resistance to some commonly used antibiotics (Karlowsky et al., 2002; Johnson et al., 2007; Sherley et al., 2004). In Owerri, Nigeria, Duru et al. (2013) reported higher resistance rate of *E. coli* isolates of poultry origin to some commonly used antibiotics as obtainable in this study. Elsewhere in USA, *E. coli* isolates from poultry products have also been reported to show reduced susceptibility to some antimicrobial agents as was

obtained in this study (Johnson et al., 2007).

The *P. aeruginosa* isolates were also found to show reduced susceptibility to the tested antibiotics. It was observed in this study that the *P. aeruginosa* isolates was found to be resistant to 7(46.7%) antibiotics out of the 15 antibiotics that was used in this study. The intrinsic resistance nature of *P. aeruginosa* isolates could be responsible for the high level of resistance of the organism as seen in this study. The ability of the organism to resist the onslaught of antibiotics contributes to its pathogenicity/virulence (Seshadri and Chhatbar, 2009; Henrichfreise et al., 2007). Similarly, previous studies have also reported that *P. aeruginosa* isolates from poultry origin are multidrug resistant (Okonko et al., 2009; Seshadri and Chhatbar, 2009). The high rates of resistance found in this study can be attributed to the use of antimicrobial agents as prophylaxis, and growth promoters in poultry and other non-human purposes. Multidrug resistance bacterial pathogens isolated from poultry birds and their products have remained a significant public health concern worldwide because of their potential to cause zoonotic infections in human population. And the widespread use of antibiotics in animal-food production and other veterinary purposes have also raised several concerns related to human and animal health.

CONCLUSION

This study conclusively showed that *Salmonella* species, *E. coli* and *P. aeruginosa* isolates were the most prevalent bacteria pathogens in poultry products; and these organisms are multidrug resistant in nature. We therefore recommend the rational use of antibiotics in non-clinical purposes, and the continued detection of multidrug resistant microbes from environmental samples in order to keep the emergence and spread of resistant microbes under check.

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