



Detection of Antimicrobial Resistant *Escherichia Coli* Isolated from Swine in North-East India

KEYWORDS

Escherichia coli, antimicrobial resistance, swine, multi-drug resistance.

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ABSTRACT Antimicrobial resistance (AMR) is an ongoing concern globally. The emergence of several MDR *Escherichia coli* strains has exposed a vulnerable side of the antimicrobials. In North-East India, swine is considered an important livestock economically; therefore, the objective of the present study was to investigate the occurrence of antimicrobial resistance in healthy swine. The antimicrobial resistant characteristics of 27 *E. coli* isolates from swine (healthy) were performed by disk diffusion test. The antibiotic resistance-susceptibility profiles were conducted for 14 antimicrobials representing 9 antimicrobial drug types. The isolates had high resistance to sulphafurazole (70.37%), ampicillin (55.55%) followed by neomycin (51.85%), streptomycin (51.85%) and tetracycline (40.74%). Of the 27 *E. coli* isolates, 14 (51.85%) revealed multi-antimicrobial resistance to more than three different antimicrobial types. These results indicate an alarming percentage prevalence of multi-antimicrobial resistant *E. coli* isolates. Hence, proper maintenance should be done for curbing further problems.

Introduction:

Antimicrobials are considered important tools for treating various diseases in human and animals alike. They are also commonly used for growth promotion and prophylaxis in the rearing of farm animals. The selection pressure rendered upon using antimicrobials as feed additives or therapy has resulted in the emergence of multiple antimicrobial resistant bacteria thus making treatment difficult (Witte, 1998; van den Bogaard *et al.*, 2000; Knapp *et al.*, 2008). The emergence and dissemination of antibiotic resistant genes among bacterial strains leading to the ineffectiveness of broad spectrum antibiotic treatment is an increasing problem (Maynard *et al.*, 2003). The extensive use of such drugs leads to the inevitable selection of antimicrobial resistance (AMR) in human and animal pathogens and commensals (Catry *et al.*, 2003).

Escherichia coli is an ubiquitous commensal bacterium present in the intestinal tract of human and animals alike. Pathogenic *E. coli* are known to cause certain diseases like edema, neonatal and post-weaning diarrhea (PWD) in swine (Dho-Moulin and Fairbrother, 1999; Mainil and Daube, 2005). Therefore, antimicrobials are regularly used from time to time to cure such diseases. But the emergence of ESBL and MDR in recent times has made the usefulness of such drugs questionable.

In NorthEast India, swine is a common consumable product making it economically important. In the present study, fecal *E. coli* strains were isolated to check the susceptibility status of the various antimicrobials used for treatment as well as growth purpose.

Materials and methods:

Bacterial isolates:

A total of 28 fresh fecal samples of healthy swine were systematically collected from the farms of Meghalaya, India.

The samples were collected in swabs and placed in Stuart transport medium (Merck). Precautions were taken while handling the animals and during collection by using disposable gloves, sanitizer etc. The swabs were transported to the laboratory and were processed immediately upon arrival.

The swabs were incubated in MacConkey broth (HiMedia) 37°C for 12-18h. A loopful of the growth medium was then seeded in MacConkey Lactose Agar (HiMedia) and incubated again for 18-24h at 37°C. A suspected colony was then selected for Gram staining and various tests such as IMViC (indole, methyl red, Voges Proskauer and citrate), oxidase and urease. The confirmed isolates were kept in BHI (Brain Heart Infusion) broth with 15% glycerol and maintaining the temperature at -80°C.

Antimicrobial Susceptibility testing:

All the *E. coli* isolates obtained were subjected to the antimicrobial susceptibility test. The test was done as per recommendations of Clinical Laboratory Standards Institute (CLSI, 2011). The test was performed on Mueller-Hinton Agar (HiMedia, Mumbai, India). The standard strain of *Escherichia coli* (ATCC 29522) was used for quality control. The details of the commercially obtained antimicrobial discs (HiMedia, India) belonging to nine different classes are given in the Table 1.

The results obtained were then interpreted using CLSI (2011) and EUCAST (2013) guidelines. If an isolate exhibited resistance to more than three antimicrobial drugs, it was regarded as 'multi-resistant' (Tenover, 2006).

Results and discussion:

In total, 27 (96.42%) *E. coli* isolates were recovered from the 28 fecal samples collected. Most of the isolates showed resistance to sulfafurazole (70.37%), am-

picillin (55.55%), neomycin (51.85%), streptomycin (51.85%) and tetracycline (40.74%). None of the isolates registered any resistance to gentamycin, chloramphenicol, ceftazidime and ceftriaxone. All the isolates were sensitive to more than 3 antimicrobials. Chloramphenicol (92.59%) and trimethoprim-sulfamethoxazole (92.59%) were highly sensitive along with gentamycin (88.88%), co-trimoxazole (88.88%), enrofloxacin (77.77%) and ciprofloxacin (66.66%). The drugs belonging to the class cephalosporin, ceftazidime and ceftriaxone showed 66.66% and 74.07% susceptibility respectively. The comparison between the rates of resistance, intermediate and sensitivity has been shown in the bar diagram (Figure 1).

14 (51.85%) of the isolates showed multiple resistance to more than three antimicrobials. 11.11% were resistant to five, 7.47% were resistant to seven and 3.7% was resistant to 9 antimicrobials.

The results obtained from this study are in agreement with Rosengren *et al.*, 2008 where the isolates from healthy swine in Canada were resistant to tetracycline (66.8%), sulfamethoxazole (46.0%) and streptomycin (33.4%). The present isolates also showed zero susceptibility to tetracycline which is one of the most commonly prescribed first-line antimicrobial used in preventing diseases as well as for growth purpose (Roberts, 1996). The frequent and excessive use of antimicrobials has led to the increment of antimicrobial resistance (McEwen and Fedorka-Cray, 2002). A direct relationship has been found in the use of antimicrobials and antimicrobial resistance (Moniri and Dastehgoli, 2007; Miranda *et al.*, 2008; Rosengren *et al.*, 2008; Jiang *et al.*, 2011).

In conclusion, the results of this study showed that half of the *E. coli* strains obtained from the feces of healthy swine were multiple drug resistant. These findings indicate that a proper surveillance program is needed for monitoring antimicrobial susceptibility in healthy swine in the region.

Acknowledgements

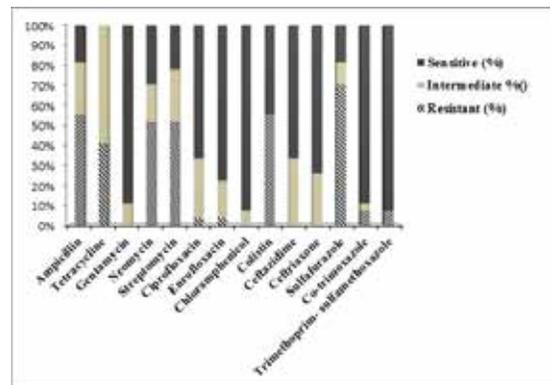
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Table and figure:

Table1: Details of the antimicrobials used in the study.

Antimicrobial Class	Antimicrobials	Disc content (µg)
Penicillin	Ampicillin	10
Cephalosporins	Ceftazidime	30
	Ceftriaxone	30
Aminoglycosides	Gentamycin	120
	Neomycin	30
	Streptomycin	25
Fluoroquinolones	Ciprofloxacin	5
	Enrofloxacin	5
Phenicol	Chloramphenicol	10
Tetracycline	Tetracycline	30
Polypeptides	Colistin	10
Sulphonamides	Sulfafurazole	300
	Co-trimoxazole	25
Trimethoprim-sulfamethoxazole		30

Figure 1: The resistance and sensitivity pattern of the E coli isolates from swine



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