Int.J.Curr.Microbiol.App.Sci (2022) *11*(02): 441-444

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 11 Number 02 (2022)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2022.1102.050

Plasmid Profile and Antibiotic Sensitivity Pattern of Porcine *Escherichia coli*

A. V. Ghodke, D. M. Muglikar[2]*, U. M. Tumlam[2], S. N. Jadhav[3] and V. V. Limaye[4]

[1]Department of A.H, Maharashtra, [2]Department of Veterinary Microbiology, [3]Department of Veterinary Biochemistry, KNP College of Veterinary Sciences, Shirval, [4]DIS Aundh, pune, Maharashtra, India

**Corresponding author*

Keywords

E.coli, diarrheic pigs, Pathogenic and non-pathogenic strains

Article Info

Received:
04 January 2022
Accepted:
31 January 2022
Available Online:
110 February 2022

A B S T R A C T

The role of plasmids in the spread of antibiotic resistance needs to be explored further in *E. coli* of porcine origin as limited no of studies have investigated the presence of plasmid and replicon types in porcine *E.coli* isolates. The prevalence of porcine *E. coli* was determined in both healthy and diarrheic pigs in the current study. The prevalence rate of 65.21% was found in diarrhoeic pigs where as only 18.36 percent of prevalence rate was observed in healthy pigs. The antmicrobial susceptibility test was conducted on 33 *E. coli* isolates. The antibiogram pattern of isolated *E.coli* revealed highest antibiotic sensitivity to Ciprofloxacin (27.27%) followed by Enrofloxacin (24.24%), where as complete resistance was observed to Amoxycillin/Cloxacillin combination. The resistance was also recorded against Penicillin (96.97%) & Ampicillin /Cloxacillin(94%). Plasmid profile analysis was carried out on 33 porcine *E.coli* isolates out of which 15 isolates revealed possession of plasmids indicating 45.45% prevalence of plasmid bearing porcine *E.coli* isolates. The variation in number and size of plasmids was found in these isolates.

Introduction

Pigs are main reservoir vessel of diverse microbes. Pathogenic and non-pathogenic strains of *Escherichia coli* are common inhabitants of the pig's intestinal tract. The use of antimicrobials as growth promoter in pig production is the main reason for persistent circulation of resistance plasmids in bacteria. Minimizing the transfer of drug resistance through plasmid is crucial for control of spread of multidrug resistant *E.coli*.

The present study highlights the prevalence and antibiotic susceptibility pattern of porcine *E. coli* in healthy and diarrheic pigs & highlights plasmid profile of these *E.coli* isolated from porcine species.

Materials and Methods

Ethical approval

All samples were collected as per standard sample collection procedure. This type of study does not require any ethical approval.

Sampling

A total of 121 faecal samples were collected from apparently healthy and diarrhoeic pigs from Pig Farm at K.N.P. College of Vet. Science, Shirwal (n=32) and a private pig farm located at Mhavshi village, taluka Khandala, district Satara (n=89). All faecal samples were collected in sterile disposable faecal collection vials. These samples were transferred immediately on ice to the lab.

Isolation and biochemical characterization of *E. Coli* isolates

All faecal samples were inoculated in nutrient broth and incubated at 37^{0C} for 24 hours. The broth culture were streaked on MacConkey agar and incubated at 37°C overnight. From each plate isolated lactose fermenting colonies were inoculated on Eosin methylene blue (EMB) agar medium for preliminary characterization. Bacterial colonies showing characteristic metallic sheen on EMB agar were picked up and considered as presumptive *E.coli*. The individual colonies were picked up and were subjected to Gram's staining. The morphology and staining reaction was noted and the single isolated colony was then transferred to Nutrient agar slant and EMB agar slants for further identification by Triple sugar iron agar & biochemical tests viz. Indole, Methyl red, Voges proskaeur and citrate utilization as per Edwards and Ewing (1972).

Antibiogram pattern and plasmid profile

The bacterial isolates were subjected to *in vitro* antibiotic sensitivity test as per the method of Bauer *et al.,* (1966). The Plasmid DNA isolation was performed by Alkaline Lysis Method with SDS as per method described by Sambrook and Russel (2001). Whereas plasmid profile analysis was done as per method described by Vandana Chaudhary (2006)

Results and Discussion

In the present study, a total of 121 faecal samples (32 from K.N.P. College pig farm and 89 from pig farm at Mhavshi village) were collected to assess the prevalence of *E.coli*. The overall prevalence of *E.coli* was 27.27 % (Table 1) The data obtained in the present study was further analyzed to record the prevalence of *E.coli* in diarrheic pigs and in healthy pigs. The prevalence of *E.coli* in diarrhoeic pigs was found to be 65.21% as compared to 18.36 % in healthy pigs. (Table 1) A total of 33 *E.coli* isolates obtained from 121 faecal samples were subjected to assess the antimicrobial susceptible patterns using 8 different antibiotics. The details of antibiotics were given in (Table no 2.) The highest antibiotic sensitivity was recorded to Ciprofloxacin (27.27%) followed by Enrofloxacin (24.24%), Streptomycin (18%), and Chloramphenicol (18%),The lowest antibiotic sensitivity was observed to Ceftriaxone (12.12%), followed by Oxytetracycline (12.12.%) and Penicillin (3.03%).(Table2) Out of 33 *E.coli* only 15 isolates revealed possession of plasmids indicating 45.45% prevalence of plasmid bearing porcine *E.coli* isolates. (Table 3). All the 15 isolates revealed possession of plasmids numbering from one to seven. Different molecular size plasmids observed ranged from 1.75 kbp to 25 kbp. In all 15 *E.coli* isolates a plasmid of 25 kb was present. One isolate showed presence of 7 plasmid bands of size 25kb, 15kb, 10kb, 5kb, 3kb, 2.3kb, and 1.75kb. Other two isolates showed presence of multiple bands one with sizes 25kb, 10kb, 6kb, 3kb and other with sizes 25kb, 3kb, 2kb. These findings are in accordance with (Jan *et al.*, 2009) and they linked presence of multiple plasmids in single bacterial strain to prolong use of antibiotic drug. The antimicrobial resistance was observed in 86.67 % *E.coli* isolates bearing plasmids.

Table.1 Prevalence of porcine *E.coli* in faecal samples collected from Diarrheic and healthy pigs

Clinical status	No of faecal sample	No of *E.coli* isolates	% Positivity of *E.coli*
Healthy pigs	98	18	**18.36 %**
Diarrheic pigs	23	15	**65.21 %**
Total no	**121**	**33**	**27.27 %**

Table.2 Antimicrobial resistance of *Escherichia coli* isolated from pigs to different class of antimicrobials

Class	Antimicrobials	Amount/ disc (mg)	Diffusion zone breakpoint (mm)	S No. (%)	I No. (%)	R N0. (%)
Beta-lactamase inhibitor	Penicillin-G	10	≤ 28	1(3.03)	0(0)	**32 (96.97)**
	Ampicillin/cloxacillin	10	≤ 13	0(0.0)	2(6.06)	**31(94)**
	Ceftriaxone	30	≤ 13	4(12.12)	12(36.36)	**17 (51.52)**
Quinolones	Ciprofloxacin	10	≤ 15	9(27.27)	5(15.15)	**19 (57.58)**
	Enrofloxacin	10	≤ 15	8(24.24)	1(3.03)	**24 (72.73)**
Aminoglycosides	Streptomycin	10	≤ 11	6(18.18)	6(18.18)	**21 (63.64)**
Tetracyclines	Oxyetracycline	30	≤ 14	4(12.12)	2(6.06)	**7(81.82)**
Sulfonamides	**Chloramphenicol**	**10**	**≤ 12**	**6(18.18)**	**4(12.12)**	**21 (63.64)**

Table.3 Plasmid profile of porcine *E.coli*

Sr. no.	Sample number	Number of plasmids present	Size of Plasmid in kbp	Sr. no.	Sample number	Number of plasmids present	Size of Plasmid in kbp
1	38	-	-	18	K6	-	-
2	72	1	25	19	K4	1	**25**
3	1	1	25	20	K2	1	**25**
4	23	7	25,15,10,5,3,2.3,1.75	21	K11	1	**25**
5	K1	-	-	22	29	-	-
6	17	1	25	23	30	-	-
7	59	1	25	24	31	-	-
8	16	3	25,3,2	25	K3	-	-
9	21	1	25	26	K10	-	-
10	39	1	25	27	10	-	-
11	6	-	-	28	41	-	-
12	12	-	-	29	44	-	-
13	9	-	-	30	32	-	-
14	7	1	25	31	35	-	-
15	2	1	25	32	65	-	-
16	27	1	25	33	K12	-	
17	**K9**	**4**	**25,10,6,3**				

References

Adhikari, R. P., K. C. Kalpana, P. Shears, and A. P. Sharma (2000) Antibiotic Resistance Conferred by Conjugative Plasmid in *Escherichia coli* Isolated from Community Ponds of Kathmandu J. Health Popul Nutr.,18(1):57-59

Ateba, C. N, Bezuidenhout, C. C. (2008). Characterization of *Escherichia coli* O157 strains from humans, cattle, and pigs in the North-west Proviance, South Africa. International Journal of Food Microbiology 128: 181–188

Bauer A. W., W. M. M. Kirby, J. C. Sherries *et al.*, (1966) Antibiotic susceptibility testing. Ameican J. Pathol., 45: 493-496

Choudhary, Vandana (2006)Characterization of Escherichia coli isolates from diarrhoeic calves for transferable drug resistance ,Colicinogeny and virulence associated genes M.V.Sc. thesis submitted to Anand Agricultural University ,Anand .

Edwards,R and Ewing W.N.(1972) Identification of Enterobacteriaceae.3rd edn., Burgess publishing Co., Minosita

Nasreen Jan, MeshramS.U. Kulkarni Archana (2009) Plasmid profile anylysis of multidrug resistant E.coli isolated from UTI patients of Nagpur city,India ,5 4635-4640

Lanz, R., P. Kuhnert, and P. Boerlin. (2003) Antimicrobial resistance and resistance genes determinants in clinical *Escherichia coli* from different animal species in Switzerland. Vet. Microbiol. 91:73-84.

Lay, Khin Khin, Nisit Chansong, Rungtip Chuanchuen (2012) Plasmid Profiles of Multidrug-resistant *Escherichia coli* from Clinically Healthy Swine.Thai. J. Vet. Med., 42(2): 229-233

Lullu, P. K and Lali Growther (2012) Antibiogram and plasmid profiling of *E.coli* isolated from cattle J. Microbiol. Biotech. Res., 2 (5):801-804

Sambrook,J&D.W.Rusell (2001) Molecular cloning :Alaboratory Manual 3rd Edn.Vol.2 Cold Spring Harbor LabPress,New York.Sundari, Rupa, Jagadeesh Babu, and Siva Sankar Reddy (2012) Antibiogram of Salmonella and *E.coli* isolates obtained from pigs. Tamil Nadu J. Veterinary & Animal Sciences 8 (1) 22-25

How to cite this article:

Ghodke, A. V., D. M. Muglikar, U. M. Tumlam, S. N. Jadhav and Limaye, V. V. 2022. Plasmid Profile and Antibiotic Sensitivity Pattern of Porcine *Escherichia coli*. *Int.J.Curr.Microbiol.App.Sci.* 11(02): 441-444. doi: https://doi.org/10.20546/ijcmas.2022.1102.050