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Isolation, identification and antibiotic sensitivity pattern of *Escherichia coli* in raw pork: A cross sectional study

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Abstract
Pork is the most perishable of all important food since it contains sufficient nutrients needed to support the growth of microorganisms. Pork contains a high number of pathogenic bacteria such as *Escherichia coli*, nontyphoid *Salmonella* serovars and *Staphylococcus aureus* which remain a potential threat to human health and also serve as possible sources of infection. A wide range of possible microorganisms are transmitted by pork to human beings but fewer are likely to have a major impact on public health. In the present study, a total of 50 raw pork samples were collected from four private commercial enterprise meat outlets in Bikaner, Rajasthan and were analyzed for isolation, identification and antibiotic sensitivity pattern for *Escherichia coli*. Out of 50, 41 (82.0%) raw pork samples were positive for *Escherichia coli* (82.0%). Antibiotic sensitivity testing was performed on all the 41 *Escherichia coli* positive raw pork samples using eleven different types of antibiotics. Out of 11 antibiotics, 3 antibiotics gentamicin, streptomycin and sulphatriad showed 100% sensitivity and 2 antibiotics ampicillin and penicillin-G showed 100% resistant in all these positive *Escherichia coli* isolates.

Keywords: Pork, *Escherichia coli*, antibiotic sensitivity test, sulphatriad

Introduction

Word ‘pork has been derived from the French 'porc' and Latin 'porcus' meaning "pig". Pork has been proved to be an important source of food worldwide contributing about 40% to the total meat production around the world [1]. India possesses 9.63 million populations of swine and produces 0.333 million tons of pork which is about 5.31% of total meat production in India.

There has been an ever increasing consumption of food from animal sources in developing countries. Population, urbanization and increasing income are the basic reasons for this increasing consumption of food from animal sources. Pork is one of the oldest foods from animals. Although its consumption has not been very high in India because of religious believes but its acceptance as a food is gradually increasing as the data of 2009 and 2010 show growth in production of pork by 0.18% in Rajasthan alone [2].

Moreover, pork is excellent for muscle growth and maintenance as it contains high quality proteins, high range of unsaturated fats alongwith conjugated linoleic acid (CLA) and are an excellent source of many vitamins and minerals, including thiamine, zinc, vitamin B12, vitamin B6, niacin, phosphorus, and iron [3].

Escherichia coli remains as normal flora in the gastrointestinal tract of pigs that produce different types of enterotoxins such as heat stable toxins (ST1 and ST2), heat labile toxins (LT) and vero toxins (VT1and VT2) [4] and is recognized as an important pathogen in outbreaks of acute diarrhoea in people who consume pork especially in developing countries [5] [6]. Most of the outbreaks of *Escherichia coli* have been linked with the consumption of undercooked pork by-products; pork sausages and salami [7]. The ability of *Escherichia coli* to adapt in acidic environments has caused this microorganism to be regarded as one of the most dangerous pathogens in fermented pork products. Several studies have shown that *Escherichia coli* are able to survive the processes of fermentation, drying and storage [8].

Antimicrobial resistance in foodborne pathogens is one of the most significant concerns to human health [9, 10]. Foods of porcine origin are an important vehicle associated with development of antibiotic-resistant pathogens such as *Salmonella* spp., *Escherichia coli*, *Yersinia* spp., Staphylococci, *Listeria monocytogenes* [11].

In the recent decades, antimicrobial resistance or/and sensitivity in bacteria have been considered as a major public health problem. This is due to the fact that many of the drugs that are used to treat human infections are used in animal husbandry also as prophylactics and feed supplements, leading to selection of resistant isolates that may affect human health if they get into the food chain [12, 13].

Pork industry is in infancy in India. Due, to comparatively less consumption of pork most of the pig slaughtering is done as an unorganized sector. Such retailers have less knowledge about hygiene which can lead to cases of pork related food poisoning in the human subjects. Information and status of pork in relation to public health in this region is lacking.

Materials and Methods

The present study was conducted at the Department of Veterinary Public Health, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, India.

A total of 50 pork samples were collected from four private commercial enterprise meat outlets of Bikaner, Rajasthan. About 10-20 grams of pork samples were collected in sterilized test tubes and immediately brought to the laboratory under cold conditions. The samples were processed within 4-6 hours of collection.

Each pork sample was streaked on Nutrient agar, and MacConkey (MCA) agar plates in primary, secondary, and tertiary fashion in order to obtain isolated colonies of bacteria. These petri plates were incubated for 24 hr at 37 ºC. After 24 hr incubation these isolated colonies were cultured on Eosine Methylene blue agar (EMB) plates for isolation of *Escherichia coli* and incubated for 24 hours at 37 ºC.The growth was examined for the colonial morphology and pigmentation, and different types of colonies were sub-cultured on separate nutrient agar plates in order to obtain a pure culture.

The confirmation of the isolates as *Escherichia coli* were done using Gram's staining, Catalase test, Coagulase test, Oxidase test and a set of 12 biochemical tests provided in HiEcoli™ Identification Kit (HiMedia, Mumbai) for *Escherichia coli.* Antibiotic sensitivity test (ABST) was conducted as per the procedure prescribed by Kirby *et al* [14].

Results and Discussion

Out of 50, 41 (82.0%) raw pork samples were positive for *Escherichia coli* (figure-1). Daniyan [15] isolated *Escherichia coli* from the fresh and chopped pork samples. Anachinaba *et al* [16] also reported that *Escherichia coli* were the most common bacteria found in the pork samples. Onpirom *et al* [17] found 37.3% pork samples positive for *Escherichia coli.* Angkititrakul *et al* [18] isolated 33% *Escherichia coli* in pork samples. Yannick *et al* [19] also reported 54.4% *Escherichia coli* from pork samples. Ashraf *et al* [20] reported 73.75% pork samples found to be positive for *Escherichia coli.* Korsak *et al* [21] reported 14% samples to be positive for *Escherichia coli.* Zhao *et al* [22] isolated *Escherichia coli* (16.3%) from randomly obtained retail pork samples. Arpana *et al* [23] isolated 12.09% *Escherichia coli* in the pork samples from Guwahati and Shillong. Agerso *et al* [24] reported 15.33% pork samples to be positive for *Escherichia coli*.

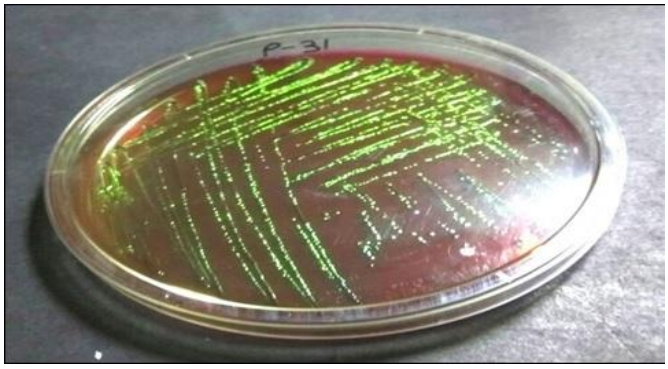

Fig 1: Isolation of *Escherichia coli* on EMB agar from Pork samples

In the present study all the isolates that were gram negative, oxidase negative, catalase positive and lactose fermenter (pink colonies) on Mac Conkey Agar were considered *Escherichia coli*. All *Escherichia coli* strains gave a green metallic sheen on EMB agar. For confirmation of *Escherichia coli* HiE.coli ™ commercial kits were used. The isolates showing a positive reaction for the Methyl red test, Indole test, Glucuronidase test, Nitrate reduction test, ONPG test, Lysine utilization test, Lactose, Glucose, Sucrose and Sorbitol sugar tests while negative with Voges proskauer's test and Citrate utilization test were confirmed as *Escherichia coli*.

The detailed results of various biochemical tests performed for *Escherichia coli* are shown in (Table-1) (Figure-2).

Table 1: Results of biochemical tests for *Escherichia coli* obtained from Hi*E.coli* ™ commercial kits

S. No.	TEST	Positive		Negative	
		Number	%	Number	%
1.	Methyl red	39/41	95.12%	02/41	04.88%
2.	Voges proskauer's	6/41	14.63%	35/41	85.37%
3.	Citrate utilization	11/41	26.83%	30/41	73.17%
4.	Indole	41/41	100.00%	-	-
5.	Glucuronidase	38/41	92.68%	3/41	07.32%
6.	Nitrate reduction	41/41	100.00%	-	-
7.	ONPG	38/41	92.68%	3/41	7.32%
8.	Lysine utilization	36/41	87.80%	5/41	12.20%
9.	Lactose	39/41	95.12%	2/41	04.88%
10.	Glucose	41/41	100.00%	-	-
11.	Sucrose	39/41	95.12%	2/41	04.88%
12.	Sorbitol	29/41	70.73%	12/41	29.27%

Variability in the results might be occurred due to presence of different types of strains of *Escherichia coli*.

Fig 2: Results of *Escherichia coli* biochemical tests for *Escherichia coli* obtained from Hi*E.coli* TM commercial kit

The present study indicated the high isolation rate of *Escherichia coli* in raw pork samples sold in Bikaner. There might be several factors responsible for that, one of the observations made during the sampling was that, in Bikaner traditional ways of slaughtering are followed for the raw pork production and most of the pork is sold by unorganized retailers where standard practices of slaughtering were not there. Polluted environment in the slaughter places and meat shops could be another source of contamination. Process of singeing, dehairing, removal of head, evisceration of the pig carcass and splitting of pig carcass were carried out at the same place which can also contribute to contamination of whole raw pork. All the offals were discarded in the waste bin placed near slaughtering point. In the last, pig carcasses were also not covered that also can lead to contamination of pork.

Antibiotic sensitivity tests were conducted against all the 41 isolates of *Escherichia coli* by using eleven different types of antibiotics that included Ampicillin, Chloramphenicol, Ciprofloxacin, Erythromycin, Gentamicin, Lincomycin, Penicillin-G, Streptomycin, Sulphatriad, Tetracycline and Vancomycin. The responses of organisms to antibiotics were categorized as sensitive, intermediate and resistant.

The antimicrobial resistance profile of the tested *Escherichia coli* isolates to different antibiotics revealed that all 41 isolates were sensitive to gentamicin, streptomycin, and sulphatriad (100%), 38 isolates were sensitive to erythromycin (92.7%), 31 isolates were sensitive to ciprofloxacin (75.6%), 22 isolates were sensitive to tetracycline (53.6%). In the present study it was also reported that all 41 isolates were found resistant to ampicillin and penicillin-G (100%), 35 isolates were found resistant to Vancomycin (85.4%). 30 isolates were found resistant to Chloramphenicol (73.2%) and 23 isolates were found resistant to Lincomycin (56.1%) (Table-2) (Figure-3).

Table 2: Antibiotic resistance profile of *Escherichia coli* isolated from pork samples

S. No	Antibiotic agent	Resistance	Intermediate resistance	Susceptible
1.	Ampicillin	41 (100%)	0 (0%)	0 (0%)
2.	Chloramphenicol	30 (73.2%)	7 (17.0%)	4 (9.8%)
3.	Ciprofloxacin	6 (14.6%)	4 (9.8%)	31 (75.6%)
4.	Erythromycin	1 (2.4%)	2 (4.9%)	38 (92.7%)
5.	Gentamicin	0 (0%)	0 (0%)	41 (100%)
6.	Lincomycin	23 (56.1%)	12 (29.3%)	6 (14.6%)
7.	Penicillin-G	41 (100%)	0 (0%)	0 (0%)
8.	Streptomycin	0 (0%)	0 (0%)	41 (100%)
9.	Sulphatriad	0 (0%)	0 (0%)	41 (100%)
10.	Tetracycline	10 (24.4%)	9 (21.9%)	22 (53.7%)
11.	Vancomycin	35 (85.4%)	2 (4.8%)	4 (9.8%)

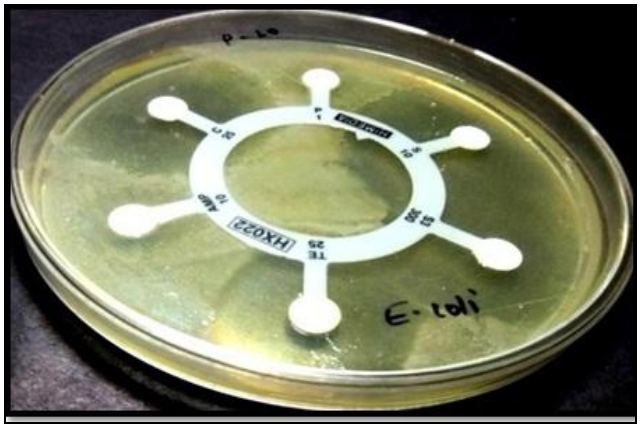

Fig 3: Antibiotic sensitivity test conducted on *Escherichia coli* isolates

Adesiji *et al* [25] reported 84.6% of *Escherichia coli* isolates from pork samples resistant to ampicillin. Further, Ali *et al* [26] reported 75% resistance to ampicillin for *Escherichia coli* while only 22.5% isolates from pork samples were reported resistant to ampicillin by Zhao *et al* [22] whereas, Jensen *et al* [27] observed the antibiotic sensitivity pattern of *Escherichia coli* and concluded that it shown the sensitivity against ampicillin (MIC >32 mg/L). Nicoline *et al* [4] observed 100% resistance to isolates. Presently, tetracycline showed 24.4% resistance to *Escherichia coli* while Zhao *et al* [22] reported that 50.3% *Escherichia coli* isolates from pork samples found to be resistant to tetracycline. Gousia *et al* [28] also reported resistance of *Escherichia coli* against tetracycline. In our study streptomycin and gentamicin showed 100% sensitivity to *Escherichia coli* means no resistance found but Zhao *et al* [22] reported that 34.6% and 18.6% *Escherichia coli* isolates from pork samples showed resistant to streptomycin and gentamicin respectively. The present findings of antibiogram revealed that the *Escherichia coli* isolates showed 14.6% resistance to ciprofloxacin whereas, Zhao *et al* [22] reported less than 5% *Escherichia coli* isolates showed resistant to ciprofloxacin. In the present study, multidrug-resistance which has been defined as resistance to 3 or more antimicrobial agents was found in most of the *Escherichia coli* isolates. In another study conducted by Zhao *et al* [22] reported the 17.3% multidrug resistance was common among *Escherichia coli* isolates.

Conclusion

The study concludes that heavy microbial load present in the pork of Bikaner city. So this requires a proper sterilization before human utilization to avoid public health problems.

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