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Original Research Article

A Bacteriological Study of Isolation of *E.coli* from Various Sources of Contamination from Food Outlets at Railway Stations of Chandigarh and Nearby Places and Its Sensitivity towards Different Antibiotics

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ABSTRACT

The aim of this study was the assessment of microbiological quality of the food serving environment at various Railway Stations of Chandigarh and nearby places, with reference to *Escherichia coli* and its isolation towards different antibiotics.
The various antibiotics used were Ampicillin, Gentamicin, Erythromycin, Chloramphenicol and Nalidixic acid. 25 samples of working area, serving area and wiping cloth were taken. Out of these 84% of the samples were considered unsafe in terms of presence of *E.coli*. This high percentage of isolation of *E.coli* clearly indicates that the environment of food cooking and serving is unsafe and unhygienic for consumption.

Keywords: *E.coli*, microbiological safety, hygiene

INTRODUCTION

Travelling by train used to be and is still one of the most accepted modes of travel. Food served and available at railway stations is being opted by a large percentage of people travelling by train. The food and the hygienic environment in which it is being served lay an impact on the overall food quality. It also emphasizes whether the food is safe for intake or not.

The food availability and official data on volume of trade involved in street vending activities is not well appreciated and maintained. Street food has a vital role in meeting the requirement of both rural and urban people.

Even though our food industry takes the initiative to feed a large percentage of population with easy accessibility and comparatively low cost food, still diseases of microbial origin are prevalent. [1] These food borne illnesses are majorly due to poor personal hygiene of vendors, inadequate storage temperatures, inappropriate methods of cooking and packaging. The reason is that these vendors lack the knowledge and are mostly uneducated about the causative factors of these diseases. They are unaware of correct techniques and at times are not licensed too to continue their food serving business. [2]

Presence of E.coli is common in food indicating prevalence of faecal pollution. [3-5] Consumers usually ignore their duty to keep in mind and check the safety and hygiene of food and are more dependent on it for convenience. [6, 7]

MATERIALS AND METHODS

In this study the microbiological quality of Food Serving environment of railway stations of Chandigarh and nearby places has been checked. A surface area measuring about 1 sq. inch was sampled with a sterilized swab from working area, serving area and the wiping cloth being used

there.

The shopkeepers and workers were interviewed regarding hygienic practices followed by them. For this purpose, a questionnaire was prepared and interviewed. Media used for cultivation of microorganisms and biochemical tests were bought from Hi-media Laboratory, Mumbai, in the dehydrated form which were reconstituted as per given instructions.

Table 1: List of Railway Stations Visited For Collection of Sample

Station No.	Place of the Railway Station
1	Kalka
2	Chandigarh
3	Khanna
4	Ambala
5	Patiala
6	Rajpura
7	Ludhiana
8	Kaurali
9	Morinda
10	Kharar

Table 2: Source of Contamination

Source of Contamination(Swabs)	Total Number
Working area	10
Serving area	10
Wiping cloth	5
Total	25

The following tests were conducted to identify *E.coli*

a) Indole production
b) Methyl red test
c) Voges Proskauer test
d) Citrate utilization test

Indole test: Principle: Indole is generated by reductive deamination from tryptophan via the intermediate molecule indole pyruvic acid. Tryptophan catalyses the deamination reaction, during which the amino group of the tryptophan molecule is removed. Final products of the reaction are indole, pyruvic acid, ammonia and energy. Pyridoxal phosphate is required as a coenzyme.

Method: Organisms were first grown in peptone water medium for 24 hours in a small tube which were incubated at 37°C. Following incubation, added 0.5ml of Kovac's reagent and the tubes shaken gently. A positive result be shown by the presence of red or red violet color on the surface of the medium.

Methyl red test: Principle: As an azodye, methyl red may be prepared by diazotization of anthranilic acid, followed by reaction with dimethylalanine. It is used to identify enteric bacteria based on their pattern of glucose metabolism. All enterics initially produce pyruvic acid from glucose metabolism. Some enterics subsequently use the mixed acid pathway to metabolize pyruvic acid to other acids. These bacteria are called methyl red positive and includes *E. coli* and *Proteus vulgaris.*

Method: The isolates were grown in tubes containing glucose phosphate, peptone water. Few drops of methyl red indicator were added to each tube and the result obtained immediately after mixing. A positive test was indicated of a bright red color, while yellow color indicates negative reaction.

Voges-Proskauer test: Principle: The test depends on the digestion of glucose to acetyl methyl carbonyl. If glucose is broken down it will react with naphthol and potassium hydroxide to form a red color. α-Naphthol and potassium hydroxide are chemicals that detect acetoin.

Method: Organisms are grown in glucose phosphate peptone water at 37°C for 24 hours. Add 0.6ml of α-naphthol solution to culture tubes followed by 0.2ml of 40% aqueous potassium hydroxide. The tubes were shaken and allowed to stand for few minutes. A positive reaction was indicated with the development of pink color within 5 minutes which later changed to crimson red after a lapse of 20 minutes.

Citrate Utilization Test: Principle: This test utilizes Kosser's liquid citrate medium to determine if a bacterium can grow utilizing citrate as sole carbon and energy source for growth.

Method: The medium prepared as slants in small tubes was stabbed with the test organisms incubated at 37°C for 24 hours. A positive reaction was represented by a change in the color of the medium from green to blue.

Preservation of Isolates

The isolated colonies were picked up with the help of inoculating needle and incubated in sterile nutrient broth. The broth was incubated for 24 hrs. The culture was streaked on nutrient agar slants and incubated at 37°C for 24 hrs.

The cultures were maintained on nutrient agar stabs, which were stored at 4°C in the refrigeration. Sub culturing was done after every 15 days.

Table 3: Sensitivity Pattern of E.coli isolates was Determined against the following Antibiotics

S.No.	Antibiotics	Concentration/disc
1	Ampicillin	35µg
2	Gentamicin	30µg
3	Erythromycin	30µg
4	Chloramphenicol	30µg
5	Nalidixic acid	30µg

Procedure

A loopful of the test organisms was transferred to 3ml sterile peptone water tubes which were incubated for 4 hrs. 0.1 ml of growth was spread on a nutrient agar plate. The antibiotics discs were then placed on the plates aseptically at equal distances and the plate was incubated at 37° C for 48 hrs. Distinct zones of inhibition of growth observed around respective disc indicated sensitivity of the organisms to the same.

RESULT

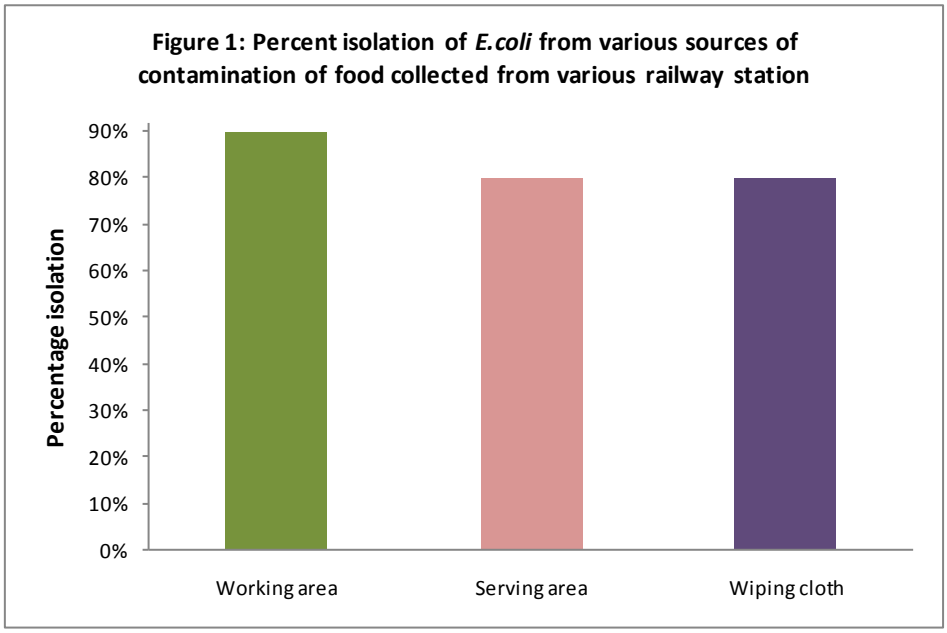
Percent isolation of *E.coli*

The presence of *E.coli* in all food samples collected from various Railway Stations and possible sources of contamination (working area, serving area and wiping cloth) and percent isolation of *E.coli* was obtained. The percent isolation of *E.coli* from ten food samples has been compared graphically.

Table 4 and Figure 1, depicts the number of *E.coli* isolated and their Percent Isolation respectively, from possible sources of contamination (working and serving area, and wiping cloth). The maximum isolation was observed in working area followed by wiping cloth and serving area.

Table 4: Isolation of *E.coli* from various sources of contamination collected from food outlets of Chandigarh Railway Station

Sample	Total No. of samples	No. of isolated *E.coli*
Working area	10	9
Serving area	10	8
Wiping cloth	5	4
Total	25	21
Percentage	100%	84%



Antibiotic sensitivity of *E.coli*:

All the strains were subjected to antibiotic sensitivity test. For these purpose five different antibiotics namely Ampicillin, Chloramphenicol, Erythromycin, Gentamicin and Nalidixic acid were used.

The results in Table 5 depict the sensitivity of *E.coli* isolated from various swab samples towards these five antibiotics. Sensitivity of 21 *E.coli* isolates from swab samples as a source of contamination towards different antibiotics is also shown in this Table. Results depict that maximum sensitivity of isolates i.e. 21 was observed towards Gentamicin, followed by 19 towards Erythromycin, 18 towards Chloramphenicol, 13 towards Nalidixic acid and a minimal towards Ampicillin.

Table 5: Sensitivity of *E.coli* isolated from swab samples towards different antibiotics

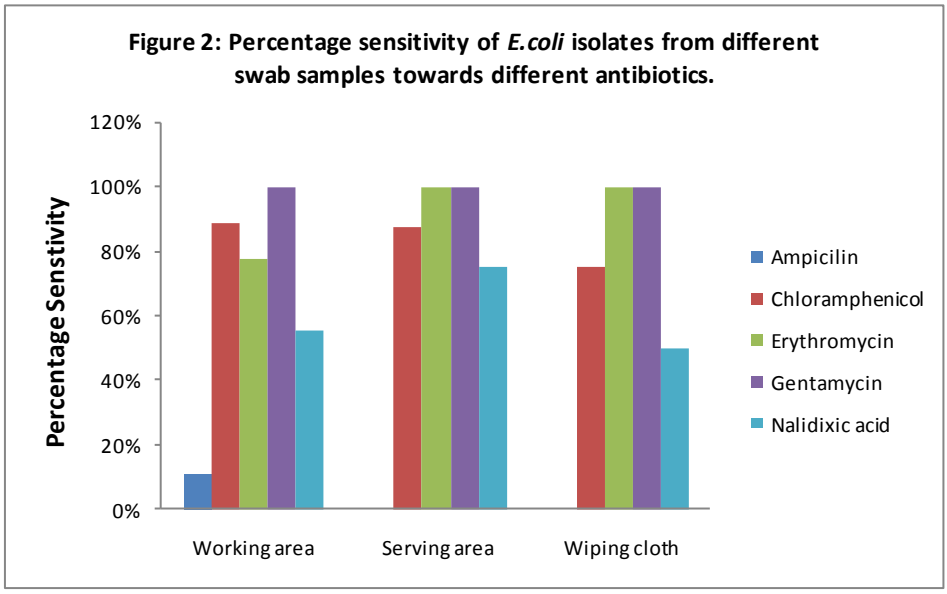
Swab Samples	Number of *E.coli* isolated	Number of isolates Sensitive to Antibiotics				
		Ampicillin	Chloramphenicol	Erythromycin	Gentamycin	Nalidixic acid
Working area	9	1	8	7	9	5
Serving area	8	-	7	8	8	6
Wiping cloth	4	-	3	4	4	2
Total	21	1	18	19	21	13
Percentage		**4.7%**	**85.7%**	**90.47%**	**100%**	**61.9%**

Figure 2 depicts the percentage sensitivity of *E.coli* isolated from the sources of contamination i.e. swab samples collected from food shops.

In the swab samples of working area, *E.coli* show maximum sensitivity towards Gentamicin (100%), followed by Erythromycin (90.47%), Chloramphenicol (85.7%), Nalidixic acid (61.9%) and least sensitive by Ampicillin (4.7%).

The *E.coli* of the serving area show maximum sensitivity towards Gentamicin and Erythromycin (100%), followed by Chloramphenicol with 88% sensitivity and 75% by Nalidixic acid.

The swabs of the wiping cloth contained *E.coli*, with 100% sensitivity to Erythromycin and Gentamicin, followed by Nalidixic acid with 80% sensitivity and 75% by Chloramphenicol. The *E.coli* shows 100% resistance to Ampicillin.



DISCUSSION

The environment around us houses a big group of microorganisms, a small portion of which are pathogenic leading to infection and disease. Foods contaminated might not always look and smell bad. At times it is impossible to determine if the food is contaminated without performing microbiological testing.

The present study was undertaken on food served at railway stations, on the various sources of contamination and microorganisms responsible for contaminating food emphasizing on

Escherichia coli as the main microbe. Samples of commonly served food items like samosa, bread pakora, tomato sauce, potato patty, potato curry, puri, kulcha, channe, burfi and water were screened for their bacterial count. The total counts of food and water samples range between 10^5 - 10^8 CFU/gm (ml) while Gram Negative counts lie between 10^4 - 10^6 CFU/gm(ml). Several authors have come up to the result that bacteria from impure dish washing waters and other sources hold on to utensil surfaces and lead to the risk of contamination during food cooking and serving . [8, 9]

Bacterial infections are usually treated with antibiotics. In response to the widespread use of antibiotics, bacteria have developed a high resistance to antibiotics. Study of sensitivity of *E.coli* strains collected towards five antibiotics is investigated.

The results indicated 100% sensitivity towards Gentamicin while water showed 88% resistance to it. Maximum sensitivity was shown by Bread Pakora, tomato sauce, potato curry and burfi isolates towards the antibiotic Chloramphenicol. In case of swab samples, all of them showed maximum sensitivity towards Gentamicin.

Proper hand hygiene is the simples and least expensive means of preventing infection, still it is not observed to be in use due to lack of knowledge and unawareness. It directly leads to health risks associated with contamination of foods by pathogenic bacteria as well as subsequent contamination by vendors during preparation and handling. [9]

In 2005 Muinde and Kuria [10] concluded that water used for preparation of foods is usually from sources that are not treated, leading to high bacterial count. The vegetables and rice used in the preparation always have contact with soil and if not properly washed with clean water could lead to a high risk for street food consumers. Earlier studies were also been shown that antibiotic susceptibility results indicated 53.85% resistance and 46.15% sensitivity among vended food isolates. The prevalence of antimicrobial resistance among food borne pathogens has increased during recent decades. [11-14]

The study had some limitations such as the sample size, which had to be limited to ten shops of railway stations. One more important factor which affected the total count was that the study was conducted in months of February and March. If the study had been conducted in summer months, the bacterial load would have been even higher.

CONCLUSION

The results clearly show that the food being consumed by a large percentage of population is not safe and not free from bacteria. The presence of bacterial pathogen *E.coli* in the food serving area, cooking area including the cloth being used by the food handler directly points out the fact that we still need to work upon the hygienic conditions and educate all the food handlers about healthy, safe and hygienic practices to be followed while preparing serving food. Education in terms of some camps, trainings or workshops can be provided to the food handlers to make them aware of the best practices and keeping our environment safe too. This will also prevent the outbreak of food borne illnesses.

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