Research Article

Detection of multi drug resistance (MDRs) strains of *Salmonella* Serovars from raw ground meat from local markets of Karachi, Pakistan: A threat for the propagation of extensively drug resistance (XDRs)

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Abstract

In developing countries, uncertain hygienic conditions play a vital in causing infection in humans & animals by contaminating food. Microbial drug resistance from perishable food items is of major concern related to foodborne illnesses. Meat is one of the foods that is heavily loaded with microbial species and can be involved in severe food infection and intoxication which includes Salmonellosis, Shigellosis, Cholera, Botulism and enteric infections. Among the induction of these drug resistant microbial strains through food; *Salmonella* species has intense role in foodborne illnesses due to contaminated meat and poultry. This current research reported the prevalence rate of enteric microbes from meat samples collected from local community markets of Karachi, Pakistan. We have found 28% of *Salmonella typhi* isolates followed by *Enterobacter aerogenes* (20%), *S. Paratyphi A* (16%), *S. Paratyphi B* (16%), *E. coli* (12%) and *Shigella dysentery* (8%). The antibiogram of the isolated strains of *S. typhi*, *S. paratyphi A* and *S. paratyphi B* reveals that a number of these strains were resistant to Chloramphenicol (C), Sulphamethoxazole (SXT), Ciprofloxacin (CIP) and Ampicillin (AMP). These resistant strains could behave as extensively drug resistance (XDRs) strains and are responsible for the major health concern regarding enteric infections in developing countries via unhygienic water and food supply.

Keywords: Antibiotic sensitivity; enteric infections; Extensively Drug Resistance (XDRs); Meat; Salmonellosis

Introduction

One of the major concerns of public health is the emergence of resistant bacterial pathogens [1-3]. These emerging strains have already been recognized as a pathogen and now transforming themselves for survival in the stressful environment and capable for foodborne transmission [4, 5]. The utilization of contaminated meat and poultry is one of the causes associated with foodborne disease. Numbers of pathogens are related to the meat and its products either fresh or frozen including strains of *Escherichia coli* significantly *Escherichia coli* O157: H7, enterohemorrhagic *E. coli* (EHEC), *L. monocytogenes*, *Staphylococcus aureus*, *Clostridium botulinum* and species of *Salmonella* [6, 7].

Among enteric spp. *Salmonella* [8] is one of the most common species responsible for foodborne illness [9]. Typhimurium and Enteritidis are the common serotypes of *Salmonella* and among them the most persistent serotype in humans is *S. Typhimurium* [4, 10, 11]. The usage of antimicrobial agents for the treatment of diseases and growth promotion of domesticated animals leads to the generation of antimicrobial resistant bacteria and their subsequent transfer to humans through the food chain [1-3]. The degree and severity of *Salmonella* resistance may vary with the type of antimicrobial agent and the geographical distribution of *Salmonella* [12]. Previous epidemiological data revealed that the foodborne pathogens particularly transmitted through raw and undercooked poultry and red meat [13].

The outbreaks of Salmonellosis are mostly related to the vast range of the food items intake from the animal source especially raw meat which is easily available at meat shops [14]. The buyer who purchases raw meat from those shops where food sanitation and its maintenance are not guaranteed got infected [15]. The infection becomes more

severe in infants, elders and those who have immune-compromised [16]. The *Salmonella* contaminated with meat at slaughterhouse results from different stages including processing, distribution, poor handling of meat, improper washing, unhygienic hands of personnel, usage of cutting boards, knives, cross contamination, and other contaminated equipment, floors, countertops, even leftover part of meat are the ways for the entrance of bacteria which causes disease [17]. Therefore this study was conducted to detect the prevalence of *salmonella* and its serovars from retail ground meat from different local markets of Karachi, Pakistan. It also correlates its resistance with the antimicrobial agent for the emergence of extremely drug resistant strains.

Materials and Methods

Meat sample collection

Samples of Raw ground meat were collected from different retail shops from local markets in Karachi, Pakistan and transported to the laboratory for analysis.

Conventional method

For Isolation and identification of *Salmonella* conventional cultural method was performed with some modifications as mentioned earlier by Rubén Robles-Reyes *et al.* [18].

Preparation of pre enrichment

1 gram of ground meat sample was weighed aseptically and transferred into the test tube. 10 ml lactose broth was added to the sample. A uniform suspension was made by blending when if necessary. Test tube were capped and left at room temperature for 1 hour. Incubation was done at 35°C for 24 ± hours.

Selective enrichment

Incubated samples were shaken gently. 1 ml of the sample was transferred to 10 ml of Selenite cystine broth. Incubation was done for 24 hours at 35°C.

Selective media plating

3mm loop full of the sample from incubated selenite cystine broth was mixed and streaked on selected media plates of nutrient agar, Salmonella Shigella agar, Bismuth Sulfite agar. Plates were incubated at 35°C for 24 to 48 hours.

Identification test

Typical colonies were picked with needle from each inoculated agar plates. Each colony was inoculated into triple sugar iron (TSI) agar Slants and Citrate slants. Incubation in TSI and Citrate slants was done at 35° C for 24 hours. Acidic/Alkaline butt and slant, gas and H₂S Production was observed [18].

Antimicrobial susceptibility testing

Confirmed Salmonella and its serovars were subjected to antimicrobial susceptibility testing by disc diffusion techniques based on the CLSI guideline. A hundred strains were screened against four antimicrobial agents includes Ampicillin (10 µg), Chloramphenicol (30µg), Sulfamethoxazole Trimethoprim (25µg), ciprofloxacin (5 µg) [19].

Results and Discussion

Unhygienic conditions in developing countries play a vital role in causing variety of infections. Meat is one of the food products that is heavily loaded with microbial species and can cause severe food infection and intoxication. The emergence of extremely drug resistant *Salmonella* and its serovars from meat and its products were analyzed by a conventional method. Hundred different samples of raw meat collected from local markets of Karachi, Pakistan and processed for bacteriological isolation using the standard conventional method. In this study prevalence rate of *Salmonella* species found out as 60 % from meat samples.

The samples yield different strains of *Salmonella*. The determined percentage of

Salmonella typhi was 28%, (the highest prevalence rate), while the prevalence of *Salmonella paratyphi* A and *Salmonella paratyphi* B was 16% each. Other isolates that were also found from meat were mostly the members of the *Enterobacteriaceae* family (as shown in (Graph 1) *Escherichia coli* 12 %, *Shigella dysentery* 08% and *Enterobacter aerogenes* 20%). Previous studies showed that that one of the most important causes of salmonellosis is the intake of contaminated material and *Salmonella typhi* is the most frequently identified serovar from a diarrheal outbreak in humans and animals [20].

Multiple steps can favor the contamination of Food with isolated pathogens including processing, distribution, handling or preparation of the meat. Various epidemiological data revealed that the food of animal origin as the major vehicle associated with illnesses caused by food-borne pathogens [21, 22].

For antimicrobial susceptibility testing, different commercially available antibiotics were used against *Salmonella* serovars and found that these strains showed resistance against them as shown in (Graph 2-4). An increase number of *Salmonella* and its serotypes showed resistance against used antimicrobial drugs like chloramphenicol, Ciprofloxacin, sulfamethoxazole, and ampicillin. Resistance towards Ampicillin (AMP) and chloramphenicol is associated with a plasmid [23] as *Salmonella* serovars are also known for plasmid-mediated virulence and resistance having plasmids of varying ranges.

The spread of *Salmonella* and its serotypes having plasmid to other humans and animals is a serious issue. The investigation should be made on the relationship of these plasmids with certain drug resistance attribute and their transfer [20].

Graph 1. Prevalence of Bacterial Isolates Obtained from Raw Ground Meat

Graph 2. Antibiogram of *Salmonella typhi* strains isolated from raw ground meat

Graph 3. Antibiogram of *Salmonella paratyphi A* strains isolated from raw ground meat

Graph 4. Antibiogram of *Salmonella paratyphi B* strains isolated from raw ground meat

Conclusion

Awareness regarding the contamination problem of meat is essential because of its quality and health safety. For the possible prevention or reduction of the pathogens; constant up to date regular surveillance of the control methodologies is strongly recommended which should be applied in poultry farms, slaughterhouses and by retailers.

Authors' contributions

Conceived and designed the experiments: F Saeed & M Qudsia, Performed the experiments: M Qudsia, Analyzed the data: F Saeed, F Afaque, TA Malik & SG Nadeem, Contributed materials/ analysis/ tools: F Saeed, F Afaque, TA Malik & SG Nadeem, Wrote the paper: F Saeed, M Qudsia & TA Malik.

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