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Research Article

STUDIES ON INCIDENCE AND ANTIBIOGRAM OF *SALMONELLA* SEROVARS ISOLATED FROM RAW PORK IN AIZAWL AND IMPHAL, INDIA

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ABSTRACT: The study was conducted to determine the incidence, serotypes and antimicrobial resistance profile of *Salmonella* serovars isolated from raw pork of Aizawl and Imphal, India. A total of 200 raw pork samples (100 from Aizawl and Imphal each) were collected from unorganized butcher shops and subjected for isolation and identification of *Salmonella* spp. which phenotypically detected 5 *Salmonella* isolates. The *Salmonella* isolates were further confirmed genotypically by *16S rRNA* genus specific PCR and recorded the incidence rate of 2.50%. Serotyping of the isolates revealed that all the three isolates from Imphal were *Salmonella enterica* serovar Virchow whereas the two isolates of Aizawl were *Salmonella enterica* serovar Virchow and *Salmonella enterica* serovar Typhimurium, one each. The antimicrobial sensitivity profile against 16 antimicrobials revealed that amikacin, imipenem, ofloxacine, norfloxacin and ciprofloxacin were 100% sensitive and among the resistant antimicrobials, highest resistance was recorded against ceftriaxone (80.00%) followed by amoxyclav and cotrimoxazole (60.00% each). Presence of *Salmonella* Typhimurium from raw pork is a matter of concern from hygiene and sanitation. Moreover, the antimicrobial resistance profile showed increasing resistance against cephalosporin, amino-penicillin and amino glycosides.

Key words: Pork, Incidence, *Salmonella* serotypes, Antibiogram, Aizawl, Imphal.

INTRODUCTION

Food is the basic need for the survivability of all living organisms. Thus it acts as major route of transmission for all types of contaminants including chemical and microbiological to the consumers. Food-borne infections are the major causes of illness and death as a major public health issue all over the world and it poses subsequent economic losses (Adak *et al.* 2005).

A large proportion of the worldwide population chiefly relies on meat as a potent source of good quality protein (Bradeeba *et al.* 2013). All over the world, pork shares about 38% of meat production (Jeffries 2012). However, pork consumption varies in different parts of the world due to religious causes and unavailability of good meat producing swine breeds and poor knowledge of pig farming.

In India, pork meat is consumed in different parts and most preferred meat in north-eastern states. Most of the people in NER rear pigs as integral part of their livelihood and more than 90% consume pork (Bujarbaruah *et al.* 2006).The pig population of the North-Eastern Region shares 38% of total pig population of India with contribution of 18.77% of India's total pork production according to Livestock Census (2012) and Basic Animal Husbandry Statistics, AHDF, GOI (2013). In Manipur and Mizoram, 27.00% and 57.00% of total meat production come from pork share.

The transmission of pathogenic and virulent bacteria and the antimicrobial resistance are major concerns from public health point of view worldwide and the transmission of anti-microbial resistant bacteria is one of the burning issues for community medicine. Due to inadequate growth of organized swine industry in North-Eastern states and the back yard rearing and slaughtering practice may result in a high chance of transmission of pork borne zoonotic pathogens and the antimicrobial resistance genes from animals to human beings through raw pork due to abuse of antibiotics.Such indiscriminate use of antimicrobial agents pressurizes the commensal and pathogenic microorganisms and clonal expansion of Multi-Drug Resistant (MDR) strains of the bacteria occurs (Baquero *et al.* 1997).

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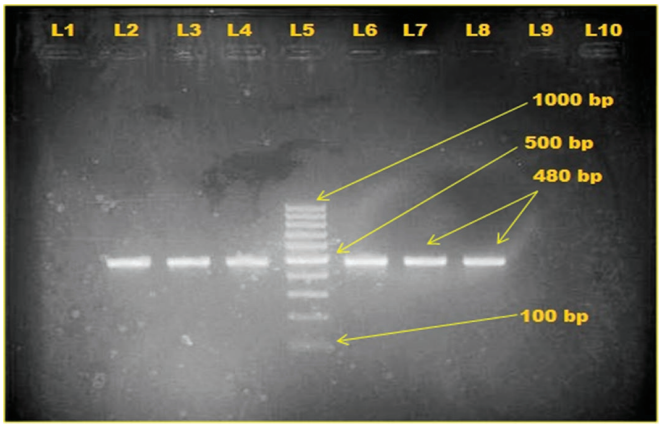

Fig. 1. Gel image of *16S rRNA* genus specific PCR for genotypic confirmation of *Salmonella* L1 Blank; L2,L3,L4, L6, L7 Samples; L8 Positive control (*Salmonella* Enteritidis - ATCC 13076); L9 Negetive control, L5 100 bp DNA ladder.

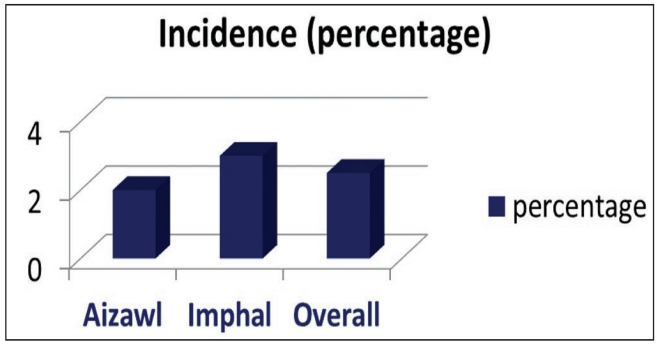

Fig. 2. Incidence of *Salmonella* in raw pork from Aizawl and Imphal.

Salmonellosis is one of the major foodborne zoonosis associated with hyperendemic diarrhoeal disease around the world affecting both man and animal alike (Prakash *et al.* 2005). Entry of these antimicrobial resistant bacteria in human food chain complicates the health issues and disease production. The incidence of zoonotic transmission of non-typhoidal *Salmonella* serovars are largely associated with food of animal origin such as eggs, milk, poultry, beef and pork meat (Alcaine *et al.* 2007, Fernandez *et al.* 2012).

The purpose of present study was to determine the incidence of and antimicrobial sensitivity profile of *Salmonella* in raw pork of two capital cities of North East India; Aizawl (Mizoram) and Imphal (Manipur).

MATERIALS AND METHODS

A total of 200 raw pork samples from different local unorganized butcher shops of Aizawl and Imphal (100 numbers from both places) were collected aseptically during the period of July 2016 to December 2016 and directly brought to the Departmental Food Safety Research Laboratory maintaining the cold chain. All the

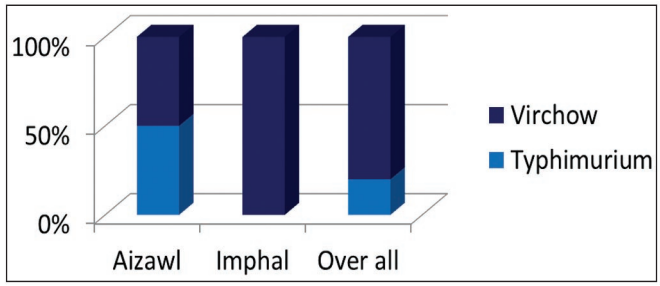

Fig. 3. Distribution of *Salmonella* serovars in Aizawl and Imphal.

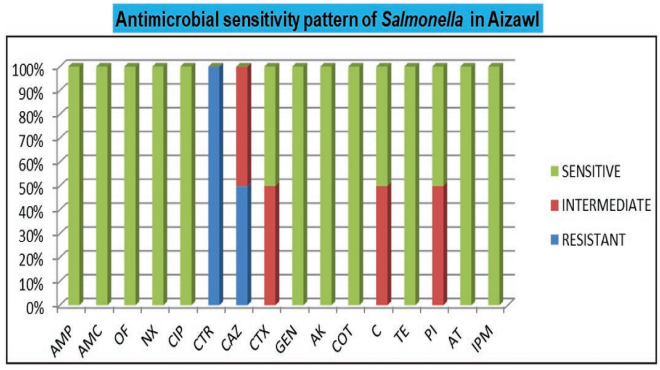

Fig. 4. Antimicrobial sensitivity pattern of *Salmonella* serovars in Aizawl.

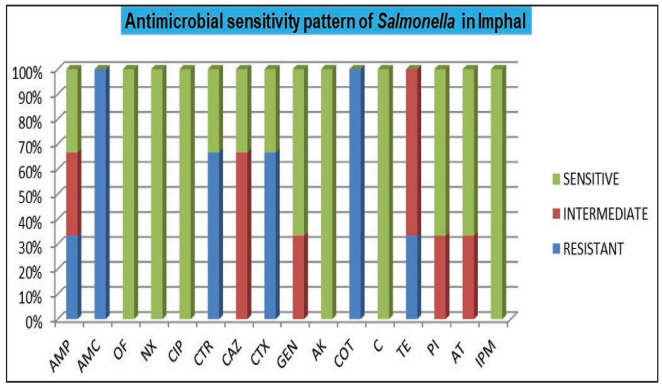

Fig. 5. Antimicrobial sensitivity pattern of *Salmonella* serovars in Imphal.

raw pork samples were processed immediately for the isolation of *Salmonella* serovars as per the standard bacteriological procedure and identification was done based on morphological and biochemical properties (Quinn *et al.* 1994). Twenty five grams of sample was weighed and placed into 225 ml 1% peptone water and incubated at 37°C for 24 hours for non-selective pre-enrichment and subsequently one ml of culture broth was inoculated in 9ml Rappaport Vassiliadis R-10 broth (RV broth) for selective enrichment of *Salmonella* and incubated at 37°C for 24 hours. The selective broth culture was inoculated in MacConkey's agar. Ten suspected pale colonies were picked and streaked on Brilliant green agar

(BGA), Xylose Lysine Deoxycholate agar (XLD) and Hektoen Enteric agar (HEA), selective agars for *Salmonella* and incubated at 37°C for 24hours. Colonies with specific colour (pink in BGA and black in XLD and HEA) were studied for their morphological characteristics and characterization of the isolates (Quinn *et al.* 1994).

All the phenotypically confirmed *Salmonella* isolates were serotyped on the basis of their somatic antigen from National *Salmonella* and *Escherichia* Centre, Central Research Institute, Kasauli, Himachal Pradesh, India.

The template DNA from suspected *Salmonella* isolate was prepared for genotypic confirmation by boiling and snap chilling method as per standard procedure. Each biochemically confirmed *Salmonella* isolate was grown in 5 ml Luria Bertani (LB) broth and incubated at 37°C overnight under constant shaking. After incubation, 1 ml of the bacterial broth culture was taken in a sterile micro-centrifuge tube and pelleted at 8000 rpm at 4°C for 10 minutes. The bacterial pellet thus obtained was washed thrice with sterile normal saline solution (NSS, 0.85% w/v) by centrifuging at 8000 rpm at 4°C for 5 minutes and finally pellet was re-suspended in 100 µl of nuclease free sterile distilled water. The bacterial suspension was boiled for 15 minutes in a boiling water bath followed by immediate chilling for 15 minutes at -20°C (Snap chilling). The lysate was centrifuged again at 5000 rpm for 5 minutes to sediment the cell debris and the supernatant was used as template DNA for PCR assay as per standard methods (Quinn *et al.* 1994).

16S rRNA genus specific PCR ligonucleotide primer (Eurofin), F: 5' - TAT CTG GCT ATC GCT GGC AGT G - 3', R: 5' - TCC GCT AAT CTT TTG GCA ACC - 3', which flank a 480 bp segment in reserved species specific gene sequence (Whyte *et al.* 2002) was used for genotypic detection of *Salmonella*. The PCR mixture included a final volume of 25 µl containing 12.5 µL 2X Dream taq PCR Master Mix $Mgcl_2$ (20 mM), dNTP mix (25 mM each)(Thermo Scientific), 1 µl (10 pmol) each of forward and reverse primer, 5 µl of template DNA (culture lysate) and nuclease free water to make up the volume 25 µl.

PCR amplification was performed in a Master Cycler Gradient (Bio Rad) at PCR cycling conditioncomprised of an initial denaturation at 95 ^{0}C for 5 minutes, followed by 30 cycles of denaturation (94 ^{0}C for 45 seconds), primer annealing (59 ^{0}C for 45 seconds) and primer extension (72 ^{0}C for 45 seconds) followed by incubation at 72 ^{0}C for 6 minutes. Finally PCR product was held in 4 ^{0}C (Whyte *et al.* 2002). The PCR amplified products were separated by horizontal submarine electrophoresis with 1% (w/v) agarose gel in 1X TAE buffer (Tris acetate 0.04 M, EDTA 0.001 M and pH adjusted to 8.0) (Sambrook *et al.* 2001).

All the genotypically confirmed *Salmonella* isolates were subjected to *in vitro* antimicrobial sensitivity test by disc diffusion against 16 commonly used antibiotics namely Ampicillin (AMP, 10), Amoxyclav (AMC, 30), Ofloxacin (OF, 5) Norfloxacin (NX, 10), Ciprofloxacin (CIP, 5), Ceftriaxone (CTR, 30), Ceftazidime (CAZ, 30), Cefotaxime (CTX, 30), Gentamicin (GEN, 10), Amikacin (AK, 30), Co-Trimoxazole (COT, 25), Chloramphenicol (C, 30), Tetracycline (TE, 30), Piperacillin (PI, 100), Aztreonam (AT, 30) and Imipenem (IPM, 10) (Hi-Media, Mumbai, India) on Muller-Hinton agar plate as per the recommendation of Clinical Laboratory Standard Institute (2014).

RESULTS AND DISCUSSION

A total of 5 *Salmonella* isolates, two from Aizawl and three from Imphal, were recovered from 200 raw pork samples indicating the incidence rates of *Salmonella* as 2.00% and 3.00%, respectively.

In this study, the serotyping results (report received from NSEC, CRI, Kasauli, Himachal Pradesh, India, showed that Salmonella isolates from Aizawl were *Salmonella enterica* serovar Typhimurium and *Salmonella enterica* serovar Virchow, one each. All of the three isolates obtained from Imphal were *Salmonella enterica* serovar Virchow.

Chaudhary *et al.* (2015) recorded 13.70% prevalence of *Salmonella* from raw pork and slaughterhouse environment in Ahmedabad, Gujrat with predominant presence of *Salmonella* Typhimurium. Das *et al.* (2012) reported 26.00% incidence of *Salmonella* in different foodstuffs with 27.77% in raw pork where as Zhao *et al.* (2001) recorded 3.30% prevalence of *Salmonella* from raw pork in a longitudinal study in and around Washington D.C. In a study conducted by Ejeta *et al.* (2004), 14.70% meat samples were contaminated with *Salmonella*. *Salmonella* was detected in 9 out of 55 pork samples (16.40%). The dominant serotype identified was *S.* infantis (36.40%).

However, Angkititrakul *et al.* (2005) reported much higher rate (65.00%) of contamination in pork samples and the most prevalent serovar in pork was *S.* Rissen (61.50%) followed by *S.* Stanley and *S.* Lexington (11.50%).The incidence of *Salmonella* was low in both the place, Aizawl and Imphal.

All the five *Salmonella* isolates showed highest sensitivity against all fluoroquinolone (ofloxacin, norfloxacin and ciprofloxacin), amikacin and imipenem (100.00%); followed by gentamicin, chloramphenicol, piperacillin and azetreonam (80.00%). Highest resistance

was showed against ceftriaxone (80.00%), followed by amoxyclav and cotrimoxazole (60.00%). Both the two *Salmonella* isolates (*S*. Typhimurium), obtained from Aizawl, were reported to be sensitive against 11 antimicrobial agents (ampicillin, amoxyclav, ofloxacin, nalidixic acid, ciprofloxacin, gentamicin, amikacin, cotrimoxazole, tetracycline, azetronam and imipenem) and resistant against ceftriaxone. However, all three *Salmonella* isolates of Imphal were found to be resistant against the same two antimicrobial agents, amoxyclav and cotrimoxazole.

Adesiji *et al.* (2014) recorded resistance to tetracycline, cotrimoxazole, nalidixic acid, nitrofurantion, and piperacillin in 66.70%, 60.00%, 53.30%, 50.00% and 50.00% of the *Salmonella* isolates obtained from human and poultry sources respectively. In a study carried (Angkititrakul *et al*. 2005) in KhonKaen, NorthEast, Thailand, all the *Salmonella* isolates from pork, chicken and human origin, were resistant to streptomycin and sulfamethoxazole. All isolates are sensitive to norfloxacin and ciprofloxacin, which was a similar pattern with the present study. With the change of source, the antimicrobial sensitivity pattern differs for the organisms. The scenario was better in the Aizawl rather than in Imphal.

CONCLUSION

The incidence rate of *Salmonella* spp. was 2.00 percent in Aizawl and 3.00 percent in Imphal from retail raw pork. This study reported *Salmonella enteric* serovar Virchow as the most prevalent serovar of Salmonella in Imphal and Aizawl, with the incidence of 80.00 percent of entire isolates. The presence of *Salmonella enterica* serovar Typhimurium was also detected. Most sensitive antimicrobials were members of fluoroquinolones, imipenem and amikacin, showing 100 percent sensitivity. The most resistant antimicrobial was ceftriaxone.

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