

# Short Research Article

## Emerging Carbapenem Resistance in *Salmonella* Species from a Tertiary Hospital in Eastern Cape, South Africa

### ABSTRACT

**Aims:** Broad-spectrum carbapenem group is the current therapy for strains of *Enterobacteriaceae* that express extended spectrum beta-lactamases (ESBLs). However, recent reports of therapeutic failures of carbapenems with strains that produce multiple  $\beta$ -lactamases are being documented. This study profiled antibiotic resistance in clinical isolates of *Salmonella* species in a tertiary hospital in the Eastern Cape of South Africa with the aim of identifying the status of *Salmonella* therapy in the region.

**Study design:** This is an analytical study.

**Place and Duration of Study:** *Salmonella* isolates (119) from blood and stool specimens of patients attending Nelson Mandela Academic Hospital Complex (NMAHC) and surrounding clinics, Eastern Cape, South Africa collected for surveillance purposes over a period of 3 years (2006 – 2009) were obtained from National Institute of Communicable Diseases, (NICD), for analysis between 2010 and 2011.

**Methodology:** Preliminary identification and serotyping were done at the NICD. The identification and antimicrobial susceptibility profile of isolates were confirmed with an Autoscan-4 antimicrobial susceptibility system. The MIC of ertapenem and imipenem tested for all *Salmonella* spp. were  $> \text{or} = 2 \text{ mg/L}$  and  $> \text{or} = 4 \text{ mg/L}$  respectively.

**Results:** A considerable portion of the isolates 59/119 (49.6 %) showed pentavalent resistance to some antibiotics including ampicillin and amoxicillin. Of the 59 multiply resistant isolates, 14 (23.7%) were resistant to 1 or more of the carbapenems examined. The phenotypic determination of ESBLs resulted in 25 (21.0 %) ESBL-positive *Salmonella* isolates. Using Fisher's exact test, the proportion of carbapenem resistance isolates was significant at  $P$  value 0.032.

**Conclusion:** The emerging resistance of *Salmonella* isolates to carbapenem drugs in this setting call for caution in usage since this is a pointer to fewer options in the choice of drugs for ESBL's therapy. Contact precautions should be put in place to forestall further transmission.

**Keywords:** Carbapenem; *Salmonella*; *Enterobacteriaceae*; resistance

### 1. INTRODUCTION

Antibiotics are often not recommended in the treatment of enteritis. It is believed that antibiotics prolong the carrier state of enteritis due to *Salmonella*. Nevertheless, systemic *Salmonella* infections and infections in the vulnerable groups qualify for antibiotic therapy [1]. These infections can be treated with ampicillin, gentamicin, trimethoprim/sulfamethoxazole, ceftriaxone, amoxicillin, or ciprofloxacin [2]. However, some *Salmonella* strains like other *Enterobacteriaceae* have become resistant not only to commonly used antibiotics but exhibit multidrug resistance. The resistance mechanism may be in the form of  $\beta$ -lactamase

production or alterations in penicillin-binding proteins (PBPs) [3]. The emergence and spread of extended-spectrum  $\beta$ -lactamases (ESBLs) - production among isolates of Enterobacteriaceae both from community and health-care settings have engendered fear. Broad-spectrum carbapenem group is the last resort therapy for strains of Enterobacteriaceae that express extended spectrum beta-lactamases [4].

Carbapenems and penems are known to possess high potency of antimicrobial activity against a broad spectrum of bacteria [4] and were known to be  $\beta$ -lactamase resistant not readily hydrolyzed by almost all  $\beta$ -lactamases, but undergo metabolism by  $\beta$ -lactamases of mammalian origin known as dehydropeptidases (DHPs) [5]. Carbapenems (doripenem, ertapenem, imipenem, meropenem) like all other  $\beta$ -lactam antibiotics (penicillins, cephalosporins, carbacephems and monobactams) have the same bactericidal mechanism of action; blocking a critical step in bacterial wall synthesis [6]. However, this currently most successful class of antibiotics is showing signs of vulnerability with recent reports of therapeutic failures of carbapenems with strains that produce multiple  $\beta$ -lactamases [7]. These bacterial strains may carry genes encoding  $\beta$ -lactamases that confer resistance to broad-spectrum  $\beta$ -lactams, including carbapenems.

The ESBL enzymes that confer resistance to extended spectrum cephalosporin and carbapenem antibiotics have over the years developed into what are known as carbapenemases. The enzymes involved in the hydrolysis of carbapenems are serine carbapenemases of the *K. pneumoniae* carbapenemase (KPC) type, of the New Delhi metallo- $\beta$ -lactamase (NDM) or Verona integron-encoded metallo- $\beta$ -lactamase (VIM) types and the imipenemase (IMP) metallo- $\beta$ -lactamase [8]. Carbapenemase resistance was also reported to have developed during ertapenem treatment of ceftriaxone-resistant and ciprofloxacin-resistant *Salmonella enterica* serotype Typhimurium [9]. Hence, *Salmonella* has been described as being very plastic in developing antimicrobial resistance [9]. The resistance determinants for carbapenem resistance frequently co-exist in mobile genetic elements (plasmids) with resistance determinants (chromosome-encoded) to other antibiotics that are commonly used against Enterobacteriaceae infections leaving few therapeutic options available [10-11]. Carbapenem resistant organisms are to be recognized as epidemiologically important and an understanding of the prevalence in their region crucial in controlling transmission [8]. This study profiled carbapenem antibiotic resistance in clinical isolates of *Salmonella* species in a tertiary hospital in the Eastern Cape province of South Africa with the aim of identifying the current state of *Salmonella* therapy in the region.

## 2. EXPERIMENTAL DETAILS

### 2.1 Study design and sampling

This is an analytical study in which *Salmonella* isolates (119) from blood and stool specimens of patients attending Nelson Mandela Academic Hospital Complex (NMAHC) and surrounding clinics deposited at the repository of the National Institute of Communicable Diseases, NICD, Johannesburg, South Africa over a period of 3 years (2006 – 2009) were obtained.

### 2.2 Bacterial isolates and characterization

The isolates which were previously identified and serotyped at the NICD were subcultured for morphological identification using standard microbiological methods. The identity and the antibiotic susceptibility pattern of the isolates were confirmed using Microscan (Siemens-

Dade Behring, South Africa). The Gram Negative Combo Panel (NUC 45) was used for the simultaneous determination of minimum inhibitory concentration of the antibiotics and ESBLs phenotypes of the isolates. The NUC 45panels contain ertapenem and imipenem at concentrations  $> \text{ or } = 2 \text{ mg/L}$  and  $> \text{ or } = 4 \text{ mg/L}$  respectively mg/l. Other types of antibiotics on the panels had concentrations (mg/L) which ranged between 0.5 and 64. The reference method was micro-broth dilution according to CLSI standards for Gram negative bacteria.

## 2.3 Statistical analysis

The results of susceptibility tests were subjected to statistical analysis using Fisher's exact test of independence, with SPSS version 18.0 (South Africa). The level of significance was set at  $P = .05$ .

## 3. RESULTS AND DISCUSSION

The study identified various serovars of *Salmonella*: *S. enterica* serovar Typhi being the highest 60/119 (51 %) followed by *S. enterica* serovar Typhimurium 30/119 (25 %), the distribution of these and other serovars is as shown (Figure 1). Resistance to five or more CLSI antibiotics subclasses was detected in 59/119 (49.6 %) of the *Salmonella* isolates. *Salmonella* isolates were resistant to amoxicillin, ampicillin, aztreonam, piperacillin/tazobactam, trimethoprim/ sulfamethoxazole and tetracycline with reduced susceptibility to ciprofloxacin. Of the 59 multiply resistant isolates, 14/59 (23.7%) exhibited resistance or intermediate resistance to at least two or all three carbapenems (ertapenem, imipenem and meropenem) drugs tested. One *Salmonella* strain was particularly resistant to all the three carbapenem drugs and all the other antibiotics except cefepime. Ten of the *Salmonella* isolates were resistant to the quinolone of choice (ciprofloxacin), besides being resistant to the second and third generation cephalosporins. Extended spectrum cephalosporins resistant phenotype was exhibited by 25/119 (21.0 %) of the *Salmonella* spp. A significant proportion of the multidrug resistant (MDR) *Salmonella* isolates obtained were ESBL-positive. This finding is consistent with the similarity in high burden of MDR strains with increasing resistance to quinolones and third-generation cephalosporins reported by Ke *et al.* [12]. A high frequency of resistance to drugs such as ampicillin, tetracycline and trimethoprim/ sulfamethoxazole was also observed in this study. However, the worrisome observation is the emerging resistance to carbapenems. The proportion of isolates resistant to carbapenem group was significant at  $P$  value .03. Increasing isolation of Gram negative bacteria resistant to carbapenem has been strongly correlated to usage of the drug in therapy [13]. Resistance to carbapenem drugs is mediated by mobile carbapenemase located on plasmids [11]. This mobile elements carriage of resistant determinants with the genetic plasticity of the *Enterobacteriaceae*, has reportedly led to rampant intra and interspecies transfer of these elements and emergence of organisms with resistance to virtually all antibiotics [14].

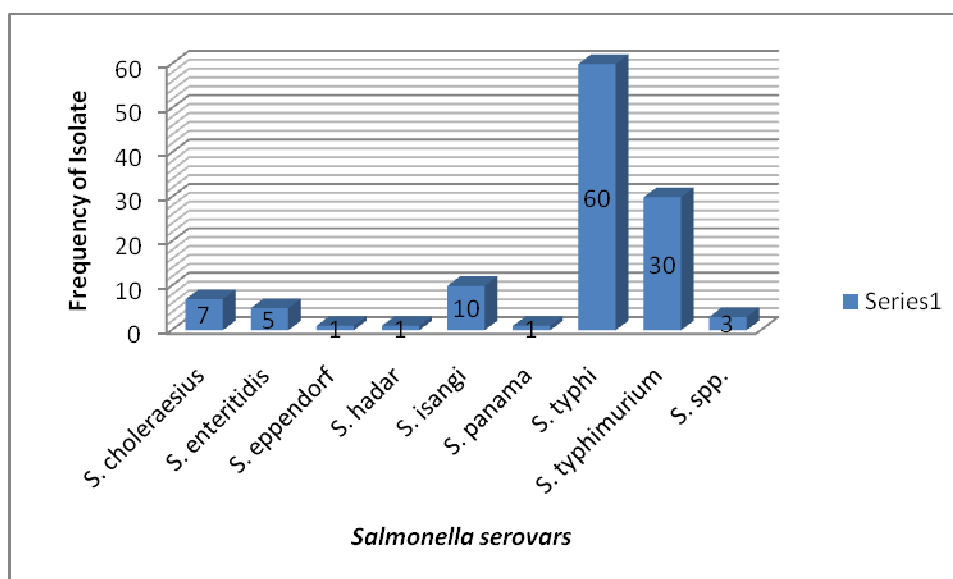


**Fig. 1. Frequency distribution of *Salmonella* serovars**

The widespread dissemination of different variants of carbapenemase has been predicted. There has been report of the spread of KPC-2 carbapenemases among *Klebsiella pneumoniae*, *E. coli*, *Salmonella* spp. and *Enterobacter* spp. in the USA [15], KPC-2- and KPC-3-producing *K. pneumoniae* in Israel [16], KPC-2 and IMP-4 in China [17]. The newest carbapenemase to emerge is New Delhi Metallo beta-lactamase 1 (NDM-1) discovered in Sweden from a patient previously hospitalized in India [10]. The NDM-1 gene produces an enzyme which makes bacteria resistant to most antibiotics (including carbapenems), except tigecycline and colistin, thus limiting the options left for the treatment of ESBLs. According to a Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) alert [18], there has been the emergence of New Delhi metallo-beta-lactamases (NDM-1) and KPC amongst hospitalized patients in South Africa and *in vivo* inter-species transfer is being envisaged. Thus, routine testing for KPCs is to be extended to all Enterobacteriaceae reported from any infected/colonized patient, and those hospitalized in the same unit [19]. South Africa is currently witnessing a spread of carbapenemases within the various provinces. The emergence of ESBL producing isolates has become a task which requires drastic interventions. Some of these isolates often are not phenotypically resistant according to CLSI guidelines with the consequential avoidable treatment failures in patients who received inappropriate antibiotic therapy [20]. Moreover, there is a need to embrace a rapid molecular diagnostic technique to detect resistant genotypes in the pathogens particularly because KPC-producers phenotypically may have carbapenem MICs below the CLSI breakpoints [18], but bearing in mind rural and resource limited settings such as this study area.

A proposed regimen to slow the development of resistance to the current armamentarium is avoiding prolonged antibiotic use or under-dosing, using pharmacokinetic and pharmacodynamic principles to choose dosing regimens, and encouraging early and aggressive empirical therapy, followed by de-escalation and narrowing the antimicrobial spectrum when culture results become available [7]. Combination therapy of rifampicin with doripenem and colistin or double carbapenem with ertapenem has been suggested [19], while fosfomycin as part of combination regimens might be useful as a last-resort option [21]. The relative safety of Carbapenems is a real advantage over the concern of selection of

142 carbapenem resistant isolates, thus there is a need to continue development of the  
143 compounds [22].

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#### 145 **4. CONCLUSION**

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147 This study reports the emergence of carbapenem resistance in clinical isolates of *Salmonella*  
148 in the Eastern Cape region of South Africa. The study showed that the emergence of  
149 carbapenem resistant *Salmonella* has serious implications in such a resource-limited  
150 hospital in sub-Saharan Africa. Given the lack of new drugs to treat these infections, efforts  
151 should be focused on infection control practices such as contact precautions.

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#### 153 **CONSENT**

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155 Bacterial isolates which originated from country surveillance repository were obtained from a  
156 secondary source not directly from patients.

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#### 159 **ETHICAL APPROVAL**

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161 The study protocol and data handling were approved by the Walter Sisulu University (WSU).  
162 Mthatha, South Africa ethical committee (protocol no. 0003/08) and the department of  
163 Health, Eastern Cape, South Africa.

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