

1 **Short Research Article**
2 **Emerging Detection of Carbapenem Resistance**
3 **in *Salmonella* Species from a Tertiary Hospital**
4 **in Eastern Cape, South Africa**

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16 **ABSTRACT**
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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 β -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 96 blood and 23 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 growing 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.

18
19 **Keywords:** Carbapenem; *Salmonella*; *Enterobacteriaceae*; resistance

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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 β -lactamase production or alterations in penicillin-binding proteins (PBPs) [3]. The emergence and spread of extended-spectrum β -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 β -lactamase resistant not readily hydrolyzed by almost all β -lactamases, but undergo metabolism by β -lactamases of mammalian origin known as dehydropeptidases (DHPs) [5]. Carbapenems (doripenem, ertapenem, imipenem, meropenem) like all other β -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 β -lactamases [7]. These bacterial strains may carry genes encoding β -lactamases that confer resistance to broad-spectrum β -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- β -lactamase (NDM) or Verona integron-encoded metallo- β -lactamase (VIM) types and the imipenemase (IMP)metallo- β -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 96 blood and 23 stool specimens of patients attending Nelson Mandela Academic Hospital Complex (NMAHC) and

70 surrounding clinics deposited at the repository of the National Institute of Communicable
71 Diseases, NICD, Johannesburg, South Africa over a period of 3 years (2006 – 2009) were
72 obtained.

73 **2.2 Bacterial isolates and characterization**

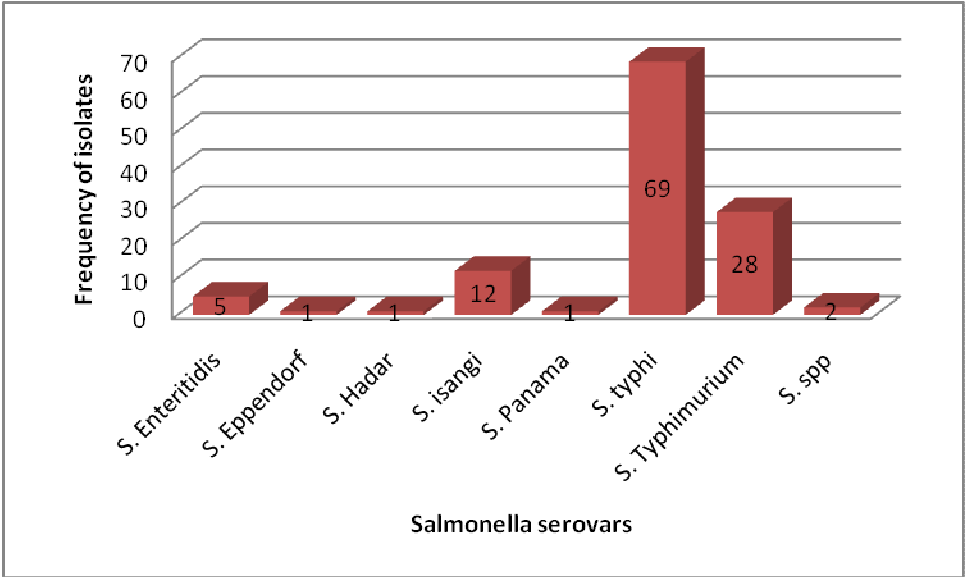
74
75 The isolates which were previously identified and serotyped at the NICD were subcultured
76 for morphological identification using standard microbiological methods. The identity and the
77 antibiotic susceptibility pattern of the isolates were confirmed using Microscan (Siemens-
78 Dade Behring, South Africa). The Gram Negative Combo 50 Panel was used for the
79 simultaneous determination of minimum inhibitory concentration of the antibiotics and ESBLs
80 phenotypes of the isolates. The Combo 50 panels contain ertapenem and imipenem at
81 concentrations ≥ 2 mg/L and ≥ 4 mg/L respectively mg/l. Other types of antibiotics on
82 the panels had concentrations (mg/L) which ranged between 0.5 and 64. The reference
83 method was micro-broth dilution according to CLSI standards for Gram negative bacteria.

84
85 **2.3 Statistical analysis**

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

89
90 **3. RESULTS AND DISCUSSION**

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92 The study identified various serovars of *Salmonella*: *S. enterica* serovar Typhi being the
93 highest 69/119 (57.9 %) followed by *S. enterica* serovar Typhimurium 28/119 (23.5 %), the
94 distribution of these and other serovars is as shown (Figure 1). Most isolates were resistant
95 to amoxicillin, ampicillin, trimethoprim/ sulfamethoxazole and tetracycline with reduced
96 susceptibility to ciprofloxacin. Resistance to five or more CLSI antibiotics subclasses was
97 detected in 59/119 (49.6 %) of the *Salmonella* isolates.



98
99 **Fig. 1. Frequency distribution of *Salmonella* serovars**

Of the 59 multiply resistant isolates, 14/59 (23.7%) exhibited resistance or intermediate resistance to at least one or two carbapenems (ertapenem, imipenem and meropenem) drugs tested. All the carbapenem-resistant isolates were serotype *S. typhi*, with one particular strain being resistant to all the three carbapenem drugs and all the other antibiotics except cefepime. Eight of the *Salmonella* isolates were resistant to the quinolone of choice (ciprofloxacin), besides being resistant to the second and third generation cephalosporins. A significant proportion of the multidrug resistant (MDR) *Salmonella* isolates obtained were ESBL-positive (Table 1). 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].

Table 1. Antibiotic resistance in *Salmonella* isolates

Salmonella serotype	No. of isolates	Resistance frequency										Tet	TmS
		AKC	Amp	Cip	Cefz	Cefu	Ctx	Cefp	Ert	Imi	Mer		
<i>S. Enteritidis</i>	5	0	3	0	2	2	2	2	0	0	0	0	0
<i>S. Eppendorf</i>	1	0	1	0	1	1	1	1	0	0	0	1	1
<i>S. Hadar</i>	1	1	1	0	1	1	1	1	0	0	0	1	0
<i>S. isangi</i>	12	12	12	2	12	12	12	12	0	0	0	12	12
<i>S. Panama</i>	1	0	0	0	0	0	0	0	0	0	0	0	0
<i>S. typhi</i>	69	27	45	6	28	63	7	5	14	5	9	44	49
<i>S. Typhimurium</i>	28	6	6	0	6	15	5	4	0	0	0	6	7
<i>S. spp</i>	2	2	2	0	1	1	1	1	0	0	0	2	2
TOTAL	119	48	69	8	51	95	29	26	14	5	9	66	71

Key: AKC – Amoxicillin/K Clavulanic acid; Amp – Ampicillin; Cip - Ciprofloxacin; Cefz – Cefazolin; Cefu – Cefuroxime; Ctx – Ceftriaxone; Cefp - Cefepime; Ert – Ertapenem; Imi – Imipenem; Mer – Meropenem; Tet – Tetracycline; TriS – Trimethoprim-Sulfamethoxazole

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 growing 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].

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

140 infected/colonized patient, and those hospitalized in the same unit [19]. South Africa is
141 currently witnessing a spread of carbapenemases within the various provinces. The
142 emergence of ESBL producing isolates has become a task which requires drastic
143 interventions. Some of these isolates often are not phenotypically resistant according to
144 CLSI guidelines with the consequential avoidable treatment failures in patients who received
145 inappropriate antibiotic therapy [20]. Moreover, there is a need to embrace a rapid molecular
146 diagnostic technique to detect resistant genotypes in the pathogens particularly because
147 KPC-producers phenotypically may have carbapenem MICs below the CLSI breakpoints
148 [18], but bearing in mind rural and resource limited settings such as this study area.

149 A proposed regimen to slow the development of resistance to the current armamentarium is
150 avoiding prolonged antibiotic use or under-dosing, using pharmacokinetic and
151 pharmacodynamic principles to choose dosing regimens, and encouraging early and
152 aggressive empirical therapy, followed by de-escalation and narrowing the antimicrobial
153 spectrum when culture results become available [7]. Combination therapy of rifampicin with
154 doripenem and colistin or double carbapenem with ertapenem has been suggested [19],
155 while fosfomycin as part of combination regimens might be useful as a last-resort option [21].
156 The relative safety of Carbapenems is a real advantage over the concern of selection of
157 carbapenem resistant isolates, thus there is a need to continue development of the
158 compounds [22].

159

160 4. CONCLUSION

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162 | This study reports the emergence detection of carbapenem resistance in clinical isolates of
163 *Salmonella* in the Eastern Cape region of South Africa. The study showed that the
164 emergence of carbapenem-resistant *Salmonella* has serious implications in such a resource-
165 limited hospital in sub-Saharan Africa. Given the lack of new drugs to treat these infections,
166 efforts should be focused on infection control practices among health-care personnel,
167 visitors and patients such as contact precautions which include patients isolation, adherence
168 to recommendations for gown and glove use by health-care workers, proper hand hygiene
169 before and after patient contact, before oral intake of drugs, food and drinks and
170 environmental sanitation.

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COMPETING INTERESTS

We declared that no competing interests exist.

AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration between both authors with joint designed of the study. Author MAB managed the literature searches, wrote the protocol, performed laboratory study, the statistical analysis and wrote the first draft of the manuscript. Author CLO managed the analyses and supervision of the study. All authors read and approved the final manuscript.

CONSENT

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

ETHICAL APPROVAL

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

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