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Antimicrobial Resistance Patterns of *Salmonella* Strains Isolated from Beef in Namibia

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Authors' contributions

This work was carried out in collaboration between all authors. Author RS designed the study, performed the statistical analysis, wrote the first draft of the manuscript and managed the literature searches. Authors RS, GK and PC managed the review of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of the study was to determine the antimicrobial resistance profiles of *Salmonella* strains isolated from beef in Namibia.
Methodology: To assess the antimicrobial resistance of *Salmonella*, a total of 81 strains isolated from 9508 routine beef samples from January 2008 to December 2009 were used. Isolation of *Salmonella* was done using a standard isolation procedure where the serotyping was done according to the White Kauffmann Le Minor scheme. *Salmonella* isolates were from carcass swabs (n = 45), meat juice (n = 19) and meat cuts (n = 17). Antimicrobial susceptibility testing on the serotyped *Salmonella* strains was carried out against 16 different antimicrobials using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar.

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Results: Twenty one of 81 isolates (25.93%) from beef samples belonging to 15 different *Salmonella* serovars showed antimicrobial resistance to one or more of the 16 antimicrobials tested and 13 (16.05%) exhibited resistance to two or more antimicrobials. Two *S.* Chester isolates and one *S.* Schwarzengrund isolate exhibited resistance to two or more antimicrobial classes. The resistance was most commonly observed to sulfisoxazole (23.46%), trimethoprim-sulfamethoxazole (13.58%), tetracycline (3.7%), amoxicillin-clavulanic acid (1.23%), cephalothin (1.23%) and chloramphenicol (1.23%). Most of the *Salmonella* isolates that showed resistance to two or more antimicrobials had a common resistance pattern to both sulfisoxazole and trimethoprim-sulfamethoxazole.

Conclusions: The present study revealed low antimicrobial resistance in *Salmonella* strains isolated from beef in Namibia. This suggests that there could still be a public health risk if such strains may reach the consumers.

Keywords: Salmonella; antimicrobials; antimicrobial resistance; beef; Namibia.

1. INTRODUCTION

Salmonella infection is a zoonotic disease which is a major challenge for both animal production and food safety. Beef and beef products can act as an important vehicle to transmit pathogens such as *Salmonella* and cause infections in humans [1]. The global *Salmonella* infections impact on public health is to the extent that there are 93.8 million cases of *Salmonella* infections and 155,000 deaths each year [2]. Considering that all *Salmonella* species are regarded as pathogenic in humans [3], the antimicrobial resistance of these bacteria may increase the severity of the disease if not carefully monitored, thus worsening the situation. One of the concerns on increasing antimicrobial resistance is that when severe infections occur, treatment with antimicrobials is difficult to achieve against the organism that is resistant to the antimicrobials used [4].

Strains of *Salmonella* with resistance to antimicrobial drugs are widespread in both developed and developing countries [5]. However, the emergence of *Salmonella* strains resistant to antimicrobials which were previously effective for treatment has placed tremendous pressure on public health systems in developing countries. The problem of antimicrobial resistance leads to the limitation on the treatment options. A few antimicrobials that are effective against these resistant pathogens are expensive and not readily available [6]. The increase in antimicrobial resistance in developing countries has been almost entirely associated with the use in human medicine while the increase in developed countries is linked with the use of antimicrobials in animal production [5]. In Africa, several studies have been carried out on the isolation of *Salmonella* serovars but few studies on *Salmonella* resistance to antibiotics have been done. Our objective was therefore to determine the antimicrobial resistance profile of *Salmonella* isolated from beef in Namibia.

2. MATERIALS AND METHODS

2.1 Sample Collection and *Salmonella* Identification

A total of 81 of *Salmonella* recovered from January 2008 to December 2009 were used in the present study. These *Salmonella* strains were isolated from three different routine beef samples; carcass swabs (n = 45), meat juice (n = 19) and meat cuts (n = 17). The isolates were recovered from 9508 routine beef samples collected from three beef export abattoirs; carcass swabs (1688), meat juice (4396) and meat cuts (3424). The isolation of *Salmonella* was done using a standard isolation procedure at the Central Veterinary Laboratory in Windhoek, Namibia. The serological identification of *Salmonella* according to the White Kauffmann Le Minor scheme [7,8] was carried out at the Istituto 'G. Caporale', Teramo, Italy. Information on the isolation, serotyping and prevalence of *Salmonella* from these three types of samples is published by Shilangale et al. [9].

2.2 Antimicrobial Susceptibility Test

Antimicrobial susceptibility test on *Salmonella* was carried out at the Istituto 'G. Caporale', Teramo, Italy. Antimicrobial susceptibility testing was done on confirmed *Salmonella* strains using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) plates [10]. Two pure *Salmonella* colonies were suspended into a tube

containing 1.5 ml saline water (0.85% NaCl). The culture suspension was adjusted against a McFarland standards (BioMérieux, Marcy-l'Étoile, France) until it achieved the turbidity of between 0.5 - 1 McFarland units. The dried surface of a Mueller-Hinton agar (Oxoid, Basingstoke, Hampshire, England) plate was inoculated by streaking the swab over the entire sterile agar surface. The resistance of *Salmonella* isolates was examined against 16 antimicrobial substances as follows: Amoxicillin-clavulanic acid (20 µg/10 µg); ampicillin (10 µg); cefazolin (30 µg); cefotaxime (30 µg); cephalothin (30 µg); chloramphenicol (30 µg); ciprofloxacin (5 µg); colistin (10 µg); enrofloxacin (5 µg); gentamicin (10 µg); kanamycin (30 µg); nalidixic acid (30 µg); tetracycline (30 µg); trimethoprim-sulfamethoxazole (1.25 µg /23.75 µg); streptomycin (10 µg); sulfisoxazole (250 µg – 300 µg). The sizes of the inhibition zones were interpreted according to the National Committee for Clinical Laboratory Standards Guidelines [11,12]. The zones diameter used to interpret as susceptible, intermediate or resistant.

3. RESULTS AND DISCUSSION

In the present study, the importance resistance to antimicrobials in Namibian *Salmonella* isolates was investigated and antimicrobial resistance pattern were generated. It was observed that 21 of 81 (25.93%) isolates from beef samples belonged to 15 different *Salmonella* serovars showed antimicrobial resistance to one or more of the antimicrobials tested (Tables 1, 2 and 3). The rest of the isolates (74.07%) were susceptible to all antimicrobials tested. However, three isolates that exhibited resistance to antimicrobials could not be identified because they could not express the phase 2 'H' antigens. Eleven (52.38%) of the isolates that showed antimicrobial resistance had antimicrobial resistant combination of sulfisoxazole and trimethoprim-sulfamethoxazole. Two isolates of *S.* Chester isolated from the carcass swabs and meat cuts and one *S.* Schwarzengrund isolated from the carcass swabs exhibited antimicrobial resistance to two or more different antimicrobial classes. The first strain of *S.* Chester isolated from carcass swabs was resistant to colistin, cephalothin, amoxicillin-clavulanic acid and tetracycline (Table 1). The other strain of *S.* Chester isolated from meat cuts showed resistance to trimethoprim-sulfamethoxazole, sulfisoxazole, tetracycline and chloramphenicol (Table 3). The only strain of *S.* Schwarzengrund isolated from carcass swabs showed antimicrobial resistance to three antimicrobials, namely; trimethoprim-sulfamethoxazole, sulfisoxazole and tetracycline (Table 1). The two *Salmonella* serovars isolated from meat juice samples showed antimicrobial resistant to two antibiotics trimethoprim-sulfamethoxazole and sulfisoxazole (Table 2).

The present study found that of the isolates that displayed resistance to antimicrobial agents tested 16.05% (N = 81) exhibiting resistance to two or more antimicrobial agents. Major serotypes that exhibited resistance were *S.* Chester (n = 6) recovered from meat cuts and carcass swabs followed by *S.* Typhimurium with 2 isolates recovered from carcass swabs while there was only 1 resistant isolate evidenced for each other identified serotype. The resistance was most commonly observed to sulfisoxazole

Table 1. Antimicrobial resistance patterns of *Salmonella* strains isolated from carcass swabs (n = 45)

Salmonella serovar	Antimicrobial resistance pattern[a]	No. of resistant strains[b]
S. Chester	CO, CE, AMC, TE	1 (6)
S. Chester	SOX	2 (6)
S. Fischerkietz	SXT, SOX	1 (1)
S. Kaapstad	SXT, SOX	1 (1)
S. Reading	CO	1 (6)
S. Saint-paul	SOX	1 (2)
S. Schwarzengrund	SXT, SOX, TET	1 (1)
S. Typhimurium	SXT, SOX	2 (5)
Salmonella enterica subsp. salamae	SOX	1 (5)
Salmonella Group C1	SOX	1 (1)
Salmonella Group D2	SXT, SOX	1 (1)

[a] SXT: trimethoprim-sulfamethoxazole; SOX: sulfisoxazole; TET: tetracycline; CST: colistin, CEF: cephalothin, AMC: amoxicillin-clavulanic acid; [b] Values in brackets shows the total number of isolates for a particular strain

(23.46%), trimethoprim-sulfamethoxazole (13.58%), tetracycline (3.7%), amoxicillin-clavulanic acid (1.23%), cephalothin (1.23%) and chloramphenicol (1.23%). Most of the *Salmonella* isolates that showed resistance to two or more antimicrobials had a common resistance pattern of trimethoprim-sulfamethoxazole and sulfisoxazole. The most notable result of this study was that 90.48% (19/21) of the resistant strains were resistant to sulfisoxazole and 52.38% (11/21) were resistant to both trimethoprim-sulfamethoxazole and sulfisoxazole.

Table 2. Antimicrobial resistance patterns of *Salmonella* strains isolated from meat juice (n = 19)

Salmonella serovar	Antimicrobial resistance pattern[a]	No. of resistant Strains[b]
S Cerro	SXT, SOX	1 (1)
S Petahtikve	SXT, SOX	1 (1)

[a] SXT: trimethoprim-sulfamethoxazole; SOX: sulfisoxazole; [b] Values in brackets shows the total number of isolates for a particular strain

Table 3. Antimicrobial resistance patterns of *Salmonella* strains isolated from meat cuts (n =17)

Salmonella serovar	Antimicrobial resistance pattern[a]	No. of resistant strains[b]
S. Anatum	SXT, SOX	1 (1)
S. Chester	SOX	1 (5)
S. Chester	SXT, SOX, TET, CL	1 (5)
S. Chester	SXT, SOX	1 (5)
S. Sao	SOX	1 (1)
S. Uganda	SXT, SOX	1 (1)

[a]SXT: trimethoprim-sulfamethoxazole; SOX: sulfisoxazole; TET: tetracycline; CL: chloramphenicol; [b] Values in brackets shows the total number of isolates for a particular strain

Similar results were observed in two different studies in Botswana where 100 % of *Salmonella* isolated from sausages, minced meat and burger patty were found to be resistant to sulfisoxazole [13] and some of the isolates from raw beef sausages were found to be resistant to trimethoprim-sulfametoxazol [14]. In these two studies, the first study did not include trimethoprim-sulfametoxazole where the latter study did not include sulfisoxazole. In a separate study in Dakar, Senegal, 14.7% of *Salmonella* isolated from beef sampled from the slaughterhouses and retailers also exhibited resistance to sulfisoxazole [15]. The findings of the present study together with the two Botswana studies suggest that trimethoprim-sulfametoxazole and sulfisoxazole could probably be the drugs of importance with regard to antimicrobial resistance of *Salmonella* in the Southern Africa. The concomitant resistance to both sulfisoxazole and trimethoprim-sulfamethoxazole was also found to be common resistance profile in the previous study of *Salmonella* isolated from feed in Namibia [16]. In this study, the prevalence of resistant *Salmonella* to this combination profile was found to be 15.5% (N = 71).

On the other hand, the level of antimicrobial resistance found in the present study was not similar to other studies done elsewhere in the Region. In Ethiopia, a study on *Salmonella* isolated from beef carcasses showed resistance mostly to ampicillin (100%), nalidixic acid and streptomycin (87.5%), tetracycline (50%) and chloramphenicol (12.5%) [17]. Unlike Namibia, the prevalence of antimicrobial resistance to tetracycline was only 3.7% with the resistance found only in S. Chester and Schwarzengrund. The study in Ethiopia did not report the characterization of *Salmonella*. Another study done on beef and meat products in Algeria found a resistance of *Salmonella* against selected 32 antimicrobials to be as much as 90.32% with 32.26% to be resistant to two or more antimicrobial agents [18]. In this study the prevalence of *Salmonella* with antimicrobial resistance to tetracycline was 12.90%. However, a study in Senegal on antimicrobial resistance of *Salmonella* isolated from beef found the resistance to tetracycline to be 0.4% [14].

When comparing the findings of the present study with others in the Region, it shows that the problem of antimicrobials resistance of *Salmonella* in Namibia is probably not as bad as it in other countries. The percentage of antimicrobial resistance and drug resistance of *Salmonella* in Namibia is lower when compared to other countries in the Region. Previous study by Shilangale et al. [16] on *Salmonella* isolated from feed in Namibia also reported low levels (19.7%) of antimicrobial resistant *Salmonella* as compared to other studies. The differences between the findings of the present study and others could be due to the differences in the geographical location [19], the type of antimicrobial agents which the animals often being exposed to and the extent of which such antimicrobial are used in food animals production

and human medicine. The current advantage of Namibia compared to other developing countries could probably be due to the strict control measures on the use of antimicrobials in both humans and animals. In Namibia antimicrobials are secured at the pharmacy or a veterinary medicine shop through a prescription from a registered medical practitioner or veterinarian. The Namibia Medicines Regulatory Council (NMRC) is a statutory body that regulates the use of medicines in Namibia. However, the data obtained in the present study is not enough to demonstrate the relationship of *Salmonella* and antimicrobial resistance in humans and food animals in Namibia. Therefore, there is a need to establish a causality relationship through epidemiological studies in this area in Namibia.

4. CONCLUSION

The problem of antimicrobial resistance in *Salmonella* has not extensively been investigated in Namibia. Although the present study found low levels of antimicrobial resistance in Namibia the availability of drug resistant *Salmonella* strains suggests that there could be a public health risk if such strains may reach the consumers. On the other hand, although the resistance to antimicrobials was observed in different *Salmonella* strains there was no relationship that could be established between the resistance to antibiotics and *Salmonella* strains. The correlation between *Salmonella* serovars with the antimicrobial resistance profile found in the present study may be useful for future studies and selection of antimicrobials for treatment of salmonellosis cases in Namibia. The fact that antimicrobial resistant *Salmonella* strains exist in Namibia mean there is a possibility that the problem may progressively grow if no new mechanisms to control the use of antimicrobials are introduced. This could happen through importation of drug resistant *Salmonella* from the neighboring countries and through tourism.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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