

Antibiotic Susceptibility and Plasmid Profiles of *Shigella* species in Sudan.

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Abstract:

This study was carried out to determine the antibiotic susceptibility, plasmid profile and conjugative abilities of *Shigella* species isolated from different towns in Sudan during 2005-2007.

Methods: Stool specimens were collected in Carry Blair transport medium from patients presenting with diarrhea from different sites in Sudan between the years 2005-2007. All specimens were inoculated on Mac Conkey's agar and Xylose Lysine Dioxycholate (XLD) (*Mast group Ltd. Merseyside U.K.*). Bacteria was isolated and subjected to different antibiotics to detect sensitivity and transference of resistance.

Results: One hundred and fourteen *Shigella* isolates were included in the study. Eighty (70.1%) were *Shigella flexeneri* representing the dominant isolate, followed by 20 (17.5%) isolates of *Shigella dysenteriae*, 9 (7.9%) *Shigella sonnei* and 5 (4.5%) *Shigella boydii*. Most of the isolates showed resistance to streptomycin (70%), tetracycline (52%) and co-trimoxazole (43%). They were highly sensitive to norfloxacin (97%), nalidixic acid (95%), gentamicin (89%) and chloramphenicol (77%). Multi-drug resistance to two or more antibiotics was apparent in most of the isolates (64, 56.1%). Fifty nine of the resistant *Shigella* isolates were studied for their ability to transfer resistance to the donor *E. coli* K₁₂ by conjugation. Of these, six were able to transfer resistance to streptomycin, tetracycline and co-trimoxazole. Extraction of the plasmid DNA from both donors and trans-conjugants showed a single type of plasmid with a molecular weight of 4.6 Kb.

Conclusion

The transfer of multi-drug resistant plasmids and the emergence of antibiotic *Shigella* and other bacterial species should raise the awareness and the seriousness of the uncontrolled (unsupervised) use of antibiotics in the medical practice.

Key words: *Shigella*, *E. coli*, Plasmid, conjugant, resistance transfer.

Diarrheal diseases constitute a major cause of morbidity and mortality among children in developing countries¹. Bacillary dysentery is one of the important causes of diarrhoea all over the world². The commonest antibiotics used for the treatment of bacillary dysentery were tetracycline and co-trimoxazole³. Multi-drug resistance to several antibiotics was reported among the different isolates of *Shigella* species in many geographic locations in the world⁴⁻⁹.

The emergence of multi-resistant plasmids in *Shigella* species has been widely recognized¹⁰⁻¹⁶. Limited studies for the antibiotic susceptibility and plasmid profile of *Shigella* species were carried in Sudan. The main objective of this research is to look for the antibiotic susceptibility and plasmid profile of *Shigella* isolates from different sites in Sudan.

Materials and Methods

Stool specimens were collected in Carry Blair transport medium from patients presenting with diarrhea from different sites in Sudan between the years 2005-2007. All specimens were inoculated on Mac Conkey's agar and Xylose Lysine Dioxycholate (XLD) (*Mast group Ltd. Merseyside U.K.*). After an overnight incubation, all non-lactose

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fermenting colonies were tested biochemically and serologically to identify the *Shigella* isolates to the species level. The antibiotic susceptibility of the isolated strains was done according to the standard NCCLS procedure¹⁷. The antibiotic discs used were tetracycline (30 µg), nalidixic acid (30 µg), co-trimoxazole (25 µg), streptomycin (30 µg), chloramphenicol (30 µg), gentamicin (20 µg) and norfloxacin (10 µg). Fifty nine isolates which showed multi-resistance were tested for their ability to transfer resistance to the recipient *E. coli* K₁₂ by conjugation according to the modified method of Ackerman and Groot¹⁸. Plasmid DNA extraction for the

donors and trans-conjugants was carried out using the modified method of Birnboim and Doly¹⁹. Gel electrophoresis and ultraviolet light were used to demonstrate the presence of the plasmid DNA and photographed by a vertical low land camera (*Polaroid No. 667*).

Results

One hundred and fourteen *Shigella* species were isolated from patients coming from different cities in Sudan. Eighty (70.1%) of them were *Shigella flexneri*, 20 (17.5%) *Shigella dysenteriae*, 9 (7.9%) *Shigella sonnei* and 5 (4.5%) *Shigella boydii*(Fig1).

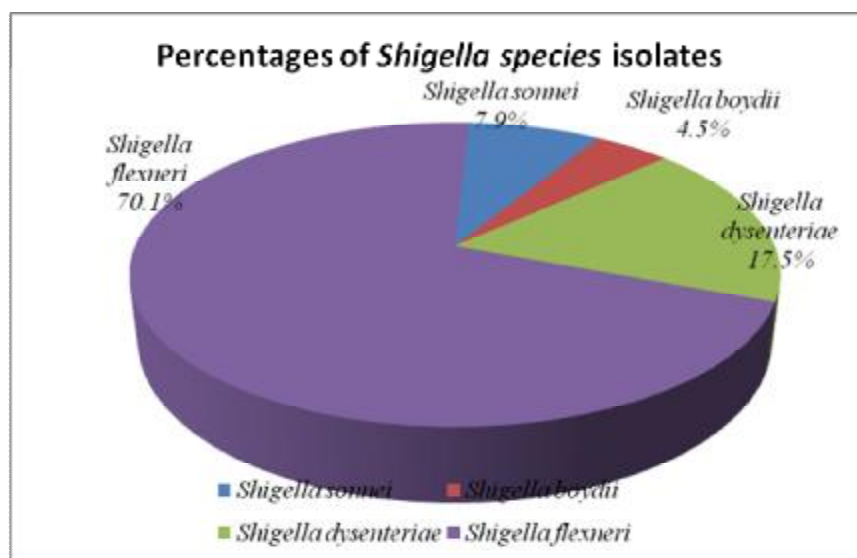


Fig. 1: Percentages of *Shigella* species isolates.

Seventy five percent of the isolates were resistant to streptomycin, 70% to tetracycline and 43% to co-trimoxazole. However, they were highly sensitive to norfloxacin (97%), nalidixic acid (95%), gentamicin (89%) and chloramphenicol (77%) (Fig.2). Multi-drug resistance was common amongst the isolates (64, 56.1%). Ten isolates were resistant to 4 antibiotics, 17 to 3 antibiotics and 37 to 2 antibiotics (Table 1). Fifty nine species of the

multi-drug resistant *Shigella* were studied for the presence of the plasmid DNA and their ability to transfer resistance genes by conjugation. Six (10.1%) of them were able to transfer the resistance plasmid to the recipient *E. coli* K₁₂. The common pattern of the resistance transferred was to: streptomycin, tetracycline and co-trimoxazole. Extraction of the plasmid DNA from both donors and recipients showed a DNA band of molecular weight 4.6 Kb (Fig. 3).

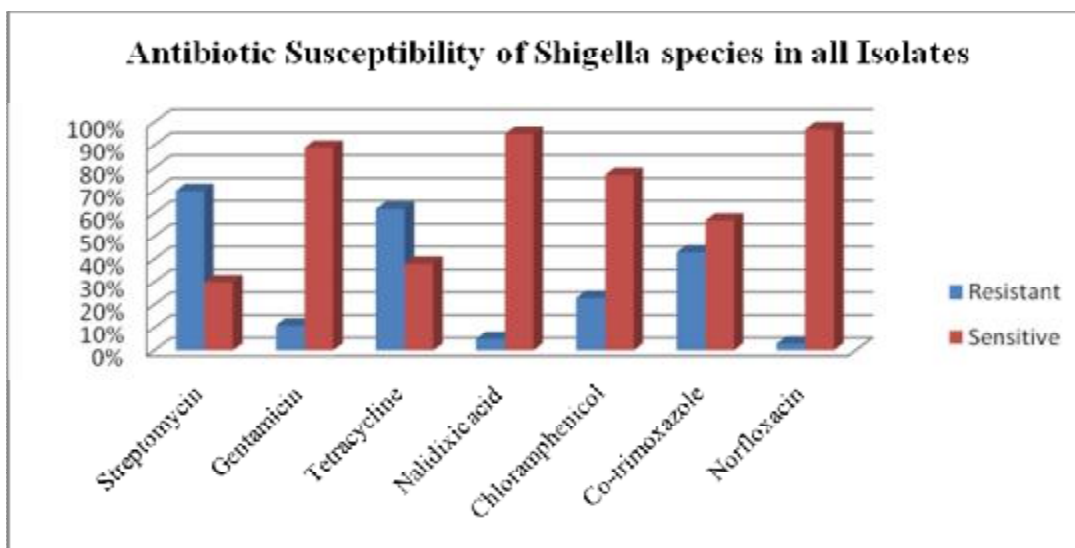


Fig 2: Antibiotic Susceptibility of *Shigella species* in all Isolates.

Table 1: Multi-resistance Isolates of *Shigella species* to different Antibiotics.

No. of Antibiotics	Pattern of Antibiotics	No. of Isolates
4	Streptomycin, Tetracycline, Chloramphenicol, Co-trimoxazole	10
3	Streptomycin, Tetracycline, Co-trimoxazole	17
2	Tetracycline, Co-trimoxazole	37

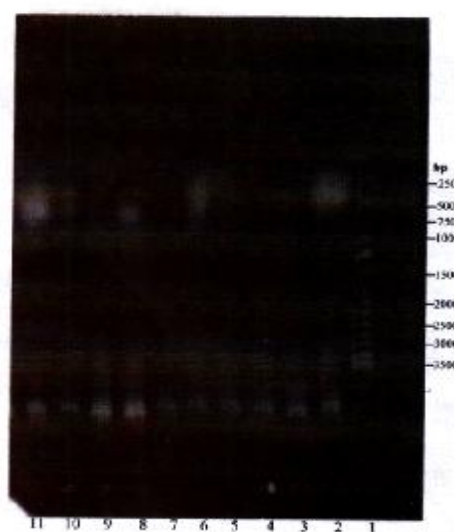


Fig. 3: Plasmid DNA extracted from donor (*Shigella species*) and recipient (*E. coli* K₁₂)
 Lane 1: DNA Marker, Lanes: 2-4-6-8-10: Donor (*Shigella species*). Lanes: 3-5-7-9-11: recipient (*E. coli* K₁₂)

Discussion

This study demonstrates the different percentages of *Shigella* species, their antibiotic susceptibility and plasmid carriage in Sudan. *Shigella flexeneri* was the dominant species (70.1%) followed by *Shigella dysenteriae*, *Shigella sonnei* and *Shigella boydii*. Similar percentages were reported from Somalia, Philippines and Egypt²⁰⁻²². However, different results were reported from Turkey and USA where the dominant species was *Shigella sonnei*²³⁻²⁴. Variation in bacterial resistance to antimicrobial agents is well known in the different geographical regions and countries²⁵. The resistance of the isolated *Shigella* species to streptomycin, tetracycline and co-trimoxazole can be explained by the overuse of those antibiotics at certain period. Similar and different antibiotic susceptibilities of *Shigella* species has been reported from various regions in the world²⁶⁻²⁷. Multiple resistances to two or more antibiotics became a common finding in most of the isolated *Shigella* species all around the world²⁴⁻²⁵. The transfer of multi-drug resistant plasmid is well recognized and reported in many published articles showing the carriage of various sizes of plasmid DNA²⁶⁻²⁷.

Conclusion

The transfer of multi-drug resistant plasmids and the emergence of antibiotic *Shigella* and other bacterial species should raise the awareness and the seriousness of the uncontrolled (unsupervised) use of antibiotics in the medical practice.

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