

### 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* K<sub>12</sub> 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 countries<sup>1</sup>. Bacillary dysentery is one of the important causes of diarrhoea all over the world<sup>2</sup>. The commonest antibiotics used for the treatment of bacillary dysentery were tetracycline and co-trimoxazole<sup>3</sup>. Multi-drug resistance to several antibiotics was reported among the different isolates of *Shigella* species in many geographic locations in the world<sup>4-9</sup>.

The emergence of multi-resistant plasmids in *Shigella* species has been widely recognized<sup>10-16</sup>. 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 procedure<sup>17</sup>. 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* K<sub>12</sub> by conjugation according to the modified method of Ackerman and Groot<sup>18</sup>. Plasmid DNA extraction for the

donors and trans-conjugants was carried out using the modified method of Birnboim and Doly<sup>19</sup>. 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).

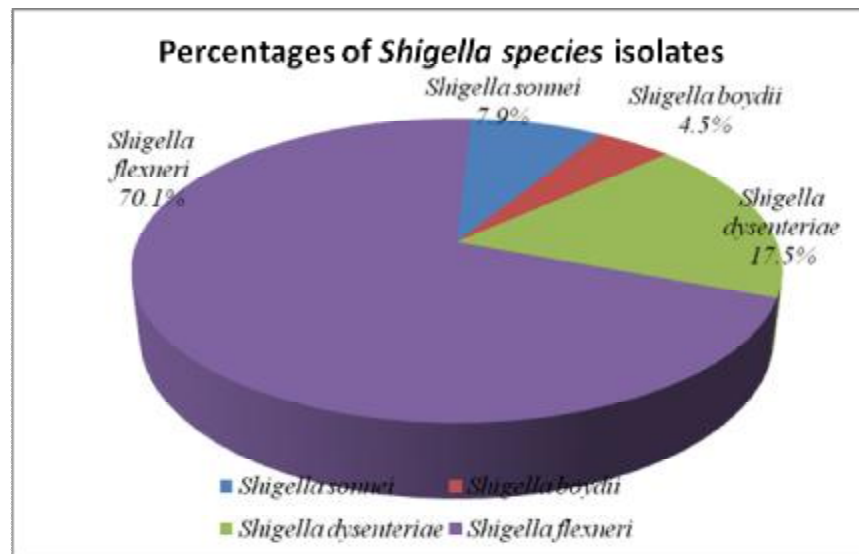


Fig. 1: Percentages of *Shigella* species isolates.

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* K<sub>12</sub>. 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).

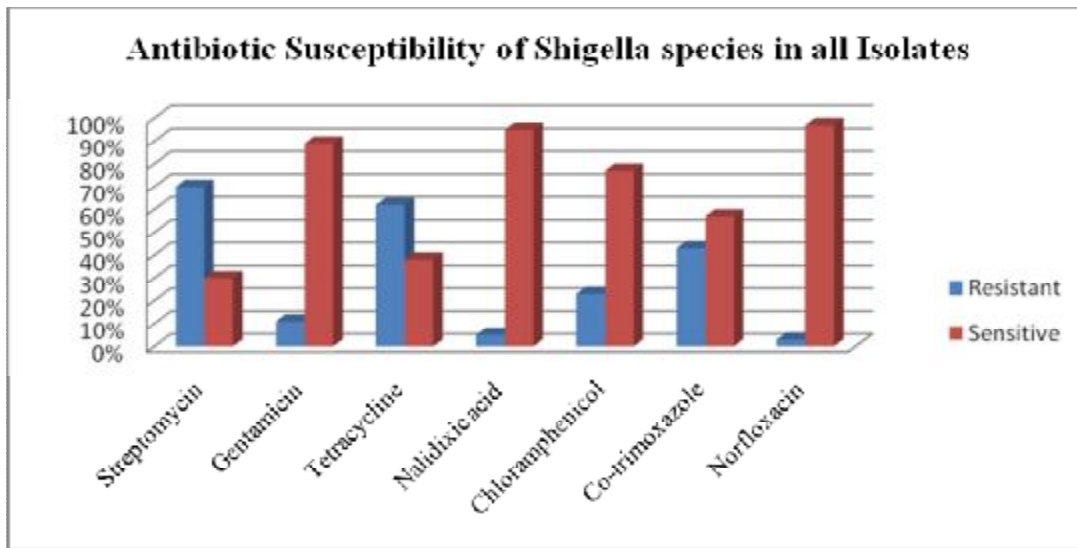


Fig 2: Antibiotic Susceptibility of *Shigella species* in all Isolates.

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

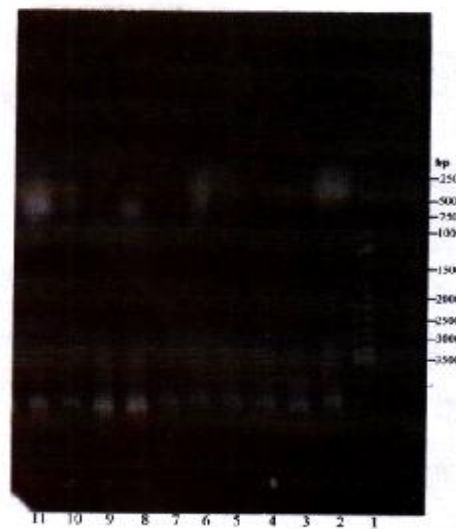


Fig. 3: Plasmid DNA extracted from donor (*Shigella species*) and recipient (*E. coli K<sub>12</sub>*)  
 Lane 1: DNA Marker, Lanes: 2-4-6-8-10: Donor (*Shigella species*). Lanes: 3-5-7-9-11: recipient (*E. coli K<sub>12</sub>*)

## 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 Egypt<sup>20-22</sup>. However, different results were reported from Turkey and USA where the dominant species was *Shigella sonnei*<sup>23-24</sup>. Variation in bacterial resistance to antimicrobial agents is well known in the different geographical regions and countries<sup>25</sup>. 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 world<sup>26-27</sup>. Multiple resistances to two or more antibiotics became a common finding in most of the isolated *Shigella species* all around the world<sup>24-25</sup>. The transfer of multi-drug resistant plasmid is well recognized and reported in many published articles showing the carriage of various sizes of plasmid DNA<sup>26-27</sup>.

## 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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