A comparative study on co-trimoxazole resistance among bacteria isolated from UTI patients and hospital wastewater

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Abstract

Antibiotic resistance, a natural phenomenon, is the ability of microorganisms to resist the effect of an antibiotic to which they were once sensitive. Resistance to first-line antibiotics and, more recently, last-line treatments, and even combination drugs could be fatal in case of minor infections. Co-trimoxazole, a combination of two antifolate compounds - sulfamethoxazole and trimethoprim, is effective against Gram-negative and Gram-positive bacteria. There are multiple reports of co-trimoxazole resistance among clinical as well as environmental bacteria making it difficult to treat bacterial infections. Horizontal gene transfer (HGT) occurs through transformation, conjugation and transduction is mainly responsible for the widespread of antibiotic resistance genes among bacterial isolates. HGT also decipher the finding of antibiotic resistant bacteria (ARB) and genes (ARG) in different environments and livestock through the transfer from environmental into the clinical pathogens. The transmission of ARGs among different bacterial species is a global human threat. This study aims at determining the prevalence and diversity of co-trimoxazole resistant bacteria in clinical and STP receiving hospital wastewater. Furthermore, this works highlights the acquisition of genetic factors viz. dfr and sul genes conferring resistance to co-trimoxazole among urinary tract infection (UTI) causing bacterial pathogens and wastewater bacterial isolates. The study will further provide in-sights into the antibiotic resistance spread in clinical and environmental samples and how these two systems interlink. A quite of few morphologically distinct bacteria (plate streaking method) or isolated from different patients showed resistance to SXT, NIT, and FOS by Kirby-Bauer Disc Diffusion Assay. Furthermore, a significant number of bacteria from clinical and environmental site were PAN drug resistant (resistance to antibiotics of all different classes).

Introduction

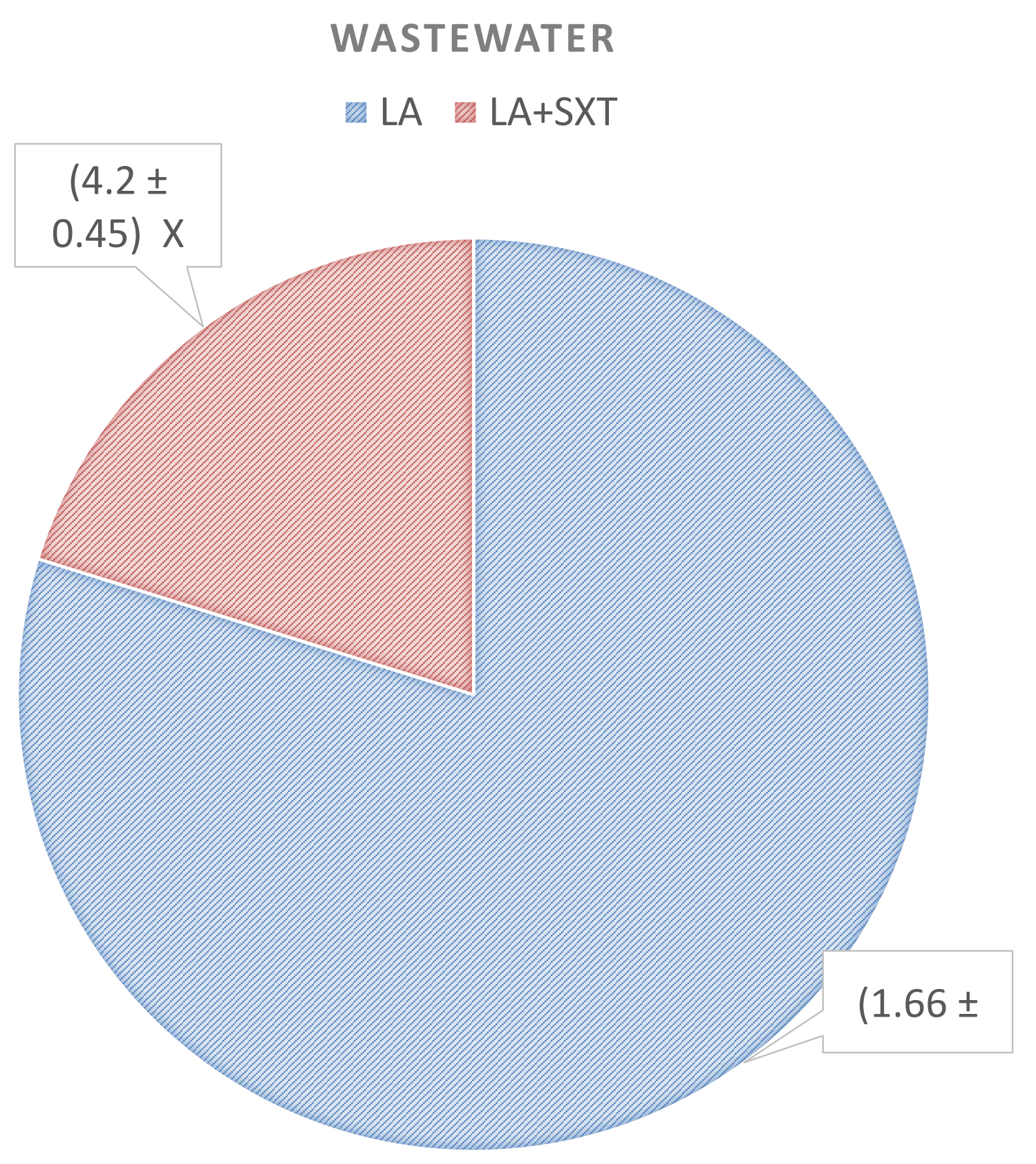
Antibiotic resistance is a property that necessitates a significant period for its global or intra or inter- species dissemination among bacteria. The drugs co-trimoxazole (COT), nitrofurantoin (NIT), and fosfomycin (FOS) are of critical importance in the therapeutic management of diverse bacterial pathogens. For the clinical samples, we chose a community hospital with a significant foot count of UTI patients throughout the year and the hospital wastewater for the environmental studies. For the bacterial load calculation,

$$\text{CFU/ml} = \frac{\text{No. of colonies X Dilution factor}}{\text{Volume taken (ml)}}$$

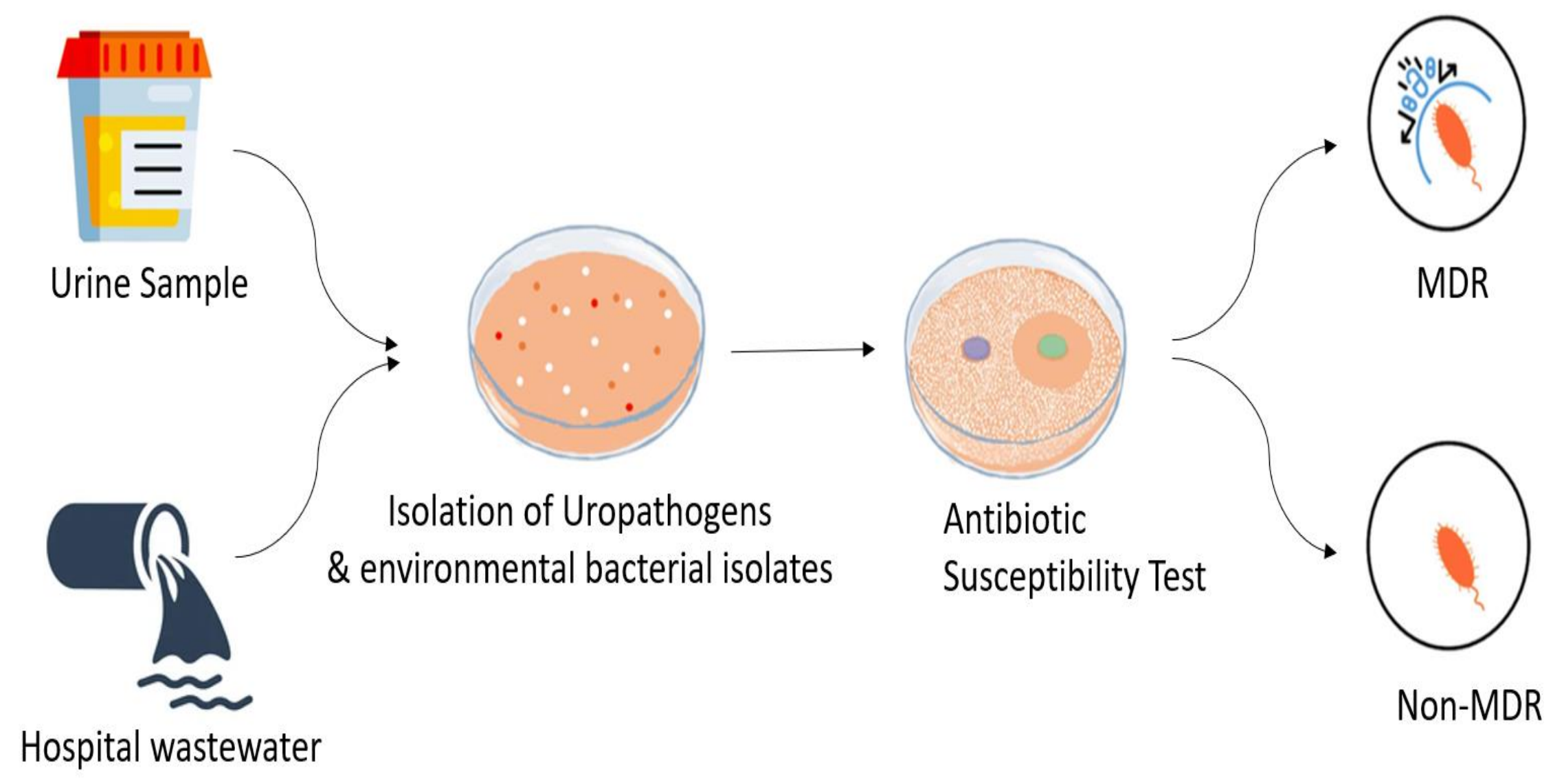
Methods and Materials

Sample collection, isolation, and phenotypic screening of bacteria resistant to co-trimoxazole

- The clinical samples (UTI pathogens on bacterial plates) was collected from a community hospital and the wastewater sample was collected in a wide-mouth polypropylene bottle and then stored immediately in an ice-box (4℃) followed by their microbiological analysis within the 18 hours.
- Isolation of bacteria was done on non-amended, or antibiotic amended CLED/ LB plates by spreading/ serial dilution plating method to calculate the bacterial load or to get the bacterial colonies.
- Morphologically distinct bacterial colonies in environmental samples were purified by streak plating method to obtain the pure bacterial isolate (Fig 3).
- Isolated pure cultures of environmental and clinical bacteria were phenotypically screened for resistance to the selected antibiotics on suitable media (Muller Hinton Agar) using disc diffusion assay as per CLSI 2023 (Fig 4).
- Bacterial colonies that were showing resistance to all three antibiotics were further screened for PAN drug resistance.

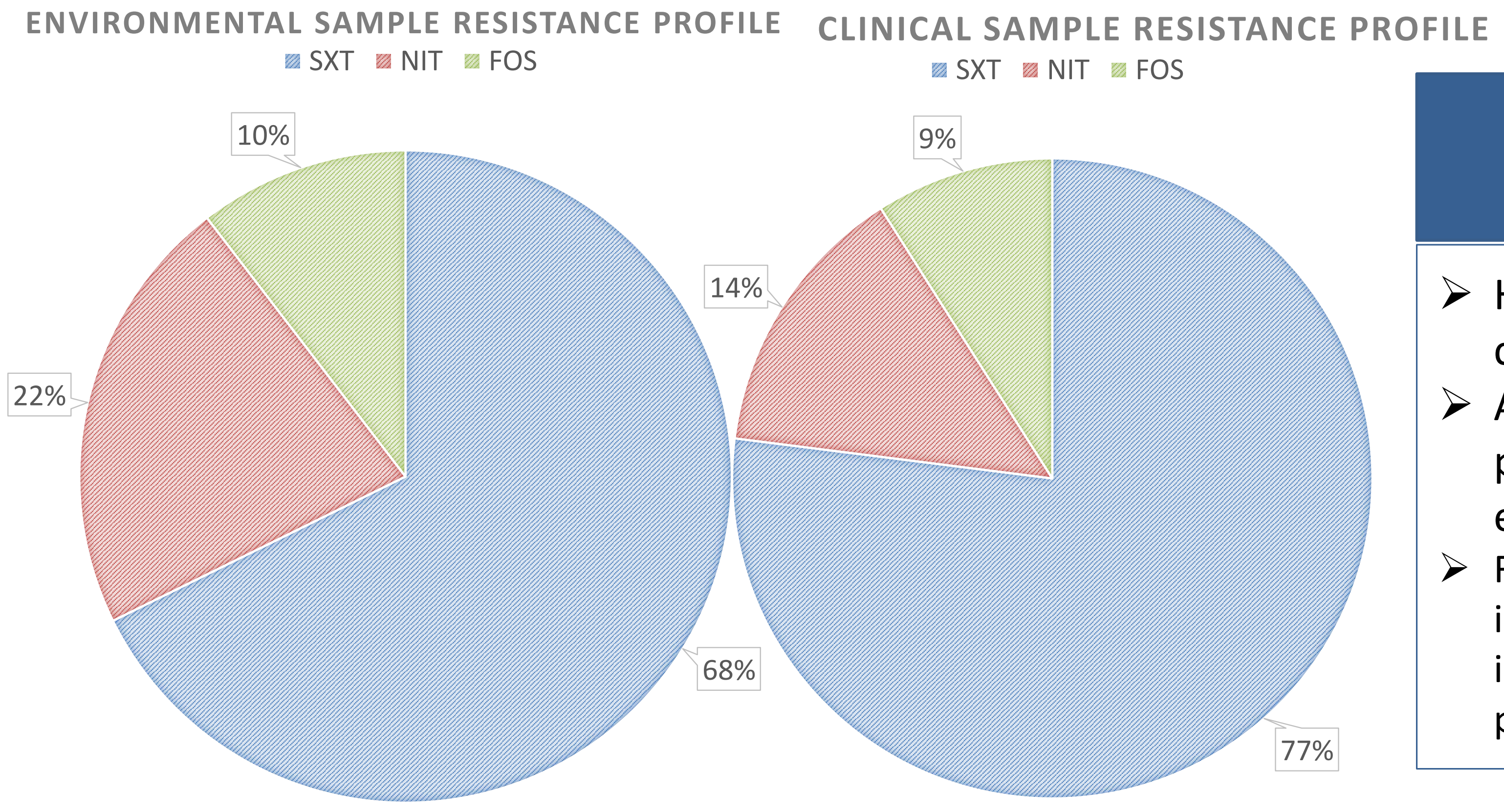


Picture 1. Bacterial load calculated on Luria Agar plate



Picture 2. Streaking of a bacterial colony

Picture 3. Phenotypic screening for resistance



Picture 4. Phenotypic antibiotic resistance profile

Results

- A high bacterial load was found in the environmental sample (Picture 1).
- A total of 57 UTI causing bacterial pathogens from different patients and 40 morphologically distinct bacteria were selected for the phenotypic screening against given antibiotics (Table).
- Six UTI causing bacteria and three environmental bacteria showed PAN drug resistance.
- No significant difference was found in the resistance percentage between clinical and environmental settings for SXT and FOS, but a significant difference is found in terms of PAN-drug resistance.

Site	Bacteria type	Number of isolates
UTI samples	Total isolates	57
	COT +ve	44
	NIT +ve	8
	FOS +ve	5
Environmental sample	Total isolates	40
	COT +ve	27
	NIT +ve	9
	FOS +ve	4

Conclusions

- High AMR bacterial load was found in clinical and environmental samples.
- A similarity in terms of resistance percentage is reported in clinical and environmental samples.
- Furthermore, how these two systems are interlinked is in our priorities as the isolated AMR bacterial isolates is preserved in glycerol at -80℃.

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References

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