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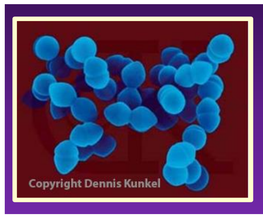

Prevalence and Characterization of Antibiotic Resistant Strains of *Enterococcus* spp. and *Acinetobacter* spp. in Community Household Environments

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BACKGROUND

The increasing prevalence of antibiotic resistance threatens the curing power of these drugs in the future. The origin of resistance is not only confined to hospital or animal agriculture environments, but our community also serves as a reservoir for several resistant bacterial strains.[2,4] Consequently, there is an upsurge in the occurrence of community-acquired infections. Members of the genera *Enterococcus* and *Acinetobacter* are included in a group called 'ESKAPE pathogens' that display a broad spectrum of antibiotic resistance and are also capable of transferring the resistance genes to other bacterial strains.[7] Several recent studies implicated their role in community-acquired infections.[1,3,5,6]

WHO Priority List 2017

Priority 1: CRITICAL[#]

- ***Acinetobacter baumannii***, carbapenem-resistant
- ***Pseudomonas aeruginosa***, carbapenem-resistant
- ***Enterobacteriaceae****, carbapenem-resistant, 3rd generation cephalosporin-resistant

Priority 2: HIGH

- ***Enterococcus faecium***, vancomycin-resistant
- ***Staphylococcus aureus***, methicillin-resistant, vancomycin intermediate and resistant
- ***Helicobacter pylori***, clarithromycin-resistant
- ***Campylobacter***, fluoroquinolone-resistant
- ***Salmonella spp.***, fluoroquinolone-resistant
- ***Neisseria gonorrhoeae***, 3rd generation cephalosporin-resistant, fluoroquinolone-resistant

ESKAPE pathogens

Enterococcus faecium (VRE)
Staphylococcus aureus (MRSA)
Klebsiella pneumonia (ESBL-producing)
Acinetobacter baumannii
Pseudomonas aeruginosa
Enterobacter species

OBJECTIVES

1. To assess the ecology and prevalence of *Enterococcus* and *Acinetobacter* antibiotic resistant strains from household environments
2. To initiate a community outreach program emphasizing cleaning protocol and stewardship on antibiotic consumption and resistance

PROCEDURE

Sample collection: Environmental samples were collected from five household items including shoe soles, restroom areas, cleaning supplies, kitchen areas, and door step/door handle. The sampling kit included pre-sterilized cotton gauze in 50 ml tube, sterilized 0.85% NaCl for dampening the gauze, and gloves for collection and instructions. A total of 30 kits were randomly distributed among undergraduate volunteers and the swabbed kits were transported back to the laboratory. **The households were located primarily in Pittsburg (26) and one in each at Frontenac, Lamar, Liberal, and Nevada.**

Isolation and characterization of bacterial isolates:

Each swab was suspended in nutrient broth & incubated at 30ºC for 24h to **enrich.** A hundred microliters of enriched broth was direct/dilution plated on to selective media: **modified *Ent.* agar (mENT)** for isolation of *Enterococcus* spp. at 44.5ºC & **modified Leeds *Acinetobacter* medium (mLAM)** for *Acinetobacter* spp. at 37ºC (Fig.1).

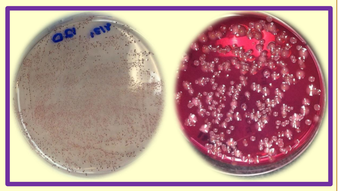

Fig.1. mENT with purple colonies of *Enterococcus and* mLAM with pink/mauve colonies of *Acinetobacter.*

PROCEDURE

Antibiotic resistance and virulence profile of bacterial isolates:

- **Genus level identification** was carried out following biochemical tests: ***Acinetobacter*** - growth on MacConkey, lactose non-fermenter (Fig.2); growth on tryptic soy agar at 44ºC; ***Enterococcus*** – Hydrolysis of esculin broth at 44ºC (Fig.3).
- **Antibiotics susceptibility** was tested by Kirby-Bauer disc diffusion method on Mueller-Hinton agar with following classes of antibiotics: β-lactams, aminoglycosides, tetracyclines, quinolones, macrolides, cephalosporins, glycopeptides, carbapenem (Fig.4). Each resistant isolate was tested for the presence of gelatinase (Fig.5) and hemolysin (Fig.7) enzyme followed by their ability to form biofilm (Fig.6).

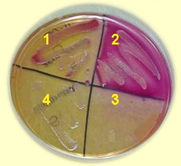

Fig.2. Screening of *Acinetobacter* on MacConkey agar

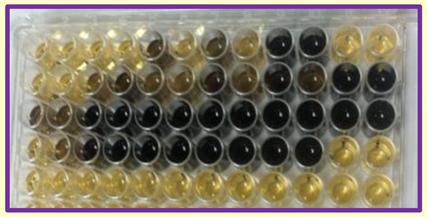

Fig.3. Esculin hydrolysis by *Enterococcus*

Fig.4. Antibiotics disc diffusion assay

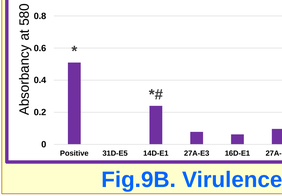

Fig.5. Gelatin hydrolysis test

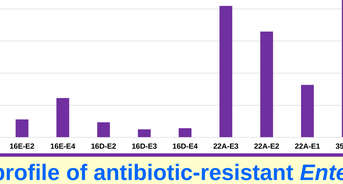

Fig.6. Biofilm assay using crystal violet

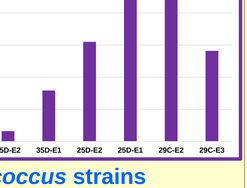

Fig.7. Hemolysin test on blood agar

RESULTS

- Selected antibiotic resistant strains were tested for various virulence characters.
- Overall, presence of gelatinase was more prevalent among *Acinetobacter* compared to *Enterococcus*. Beta-hemolysin production was rarely noted among both species.
- Two *Acinetobacter* strains harbored gelatinase, hemolysin, and were biofilm formers out of 20 tested strains (Fig.9A) while a total of 7 *Enterococcus* strains were biofilm formers and one showed hemolysin activity as well out of 20 tested strains (Fig.9B).

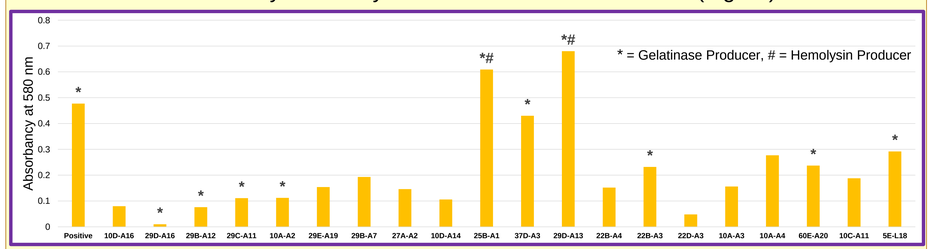

Fig.9A. Virulence profile of antibiotic-resistant *Acinetobacter* strains

Fig.9B. Virulence profile of antibiotic-resistant *Enterococcus* strains

RESULTS

- 22 out of 30 (73%) and 28/30 (93%) kits were positive for growth of *Enterococcus* spp. and *Acinetobacter* spp., respectively.
- Genus-specific identification confirmed *Acinetobacter* (140/408, 34%) and *Enterococcus* (123/172, 71%).
- **Door steps, cleaning supplies, and shoe soles (13-20%) were less frequently contaminated with enterococci compared to that of kitchen tops (16/30, 53%) and restrooms (12/30, 40%).**
- Overall, 102/150 (68%) of the swabbed surfaces were contaminated with *Acinetobacter* spp. in contrast to 43/150 (28%) with enterococci.

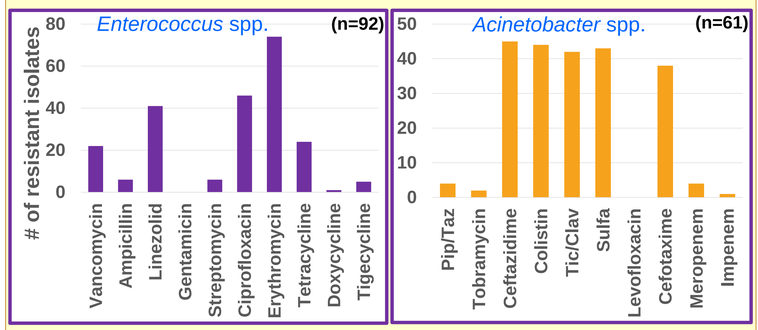

Fig.8. Antibiotic susceptibility profiles

Abbr. Pip/Taz: Piperacillin/Tazobactum; Tic/Clav: Ticaracillin/Clavulonic Acid; Sulfa: Sulfamethoxazole

- Fig. 8 shows *Acinetobacter* spp. were more frequently resistant to selected antibiotics compared to enterococci.
- Interestingly, 41 of each of *Acinetobacter* and enterococcal isolates were resistant to 3-6 antibiotics.

CONCLUSIONS & FUTURE WORK

- Our data showed that household environments were more frequently contaminated with *Acinetobacter* compared to that with *Enterococcus*.
- Multi-drug resistance was common in both species.
- *Acinetobacter* strains were more virulent compared to *Enterococcus* strains.
- **PCR amplification** of partial *sodA* gene for *Enterococcus* and partial *rpoB* gene for *Acinetobacter* are being carried out for species-level ID.

- ✓ **The community will be outreached with recommended cleaning protocol & stewardship on antibiotic consumption and resistance.**
- ✓ **The outcome of this study aims to facilitate effective and appropriate treatment options for community-acquired infections.**

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