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Resistance and virulence patterns in Gram negative and Gram positives rods isolated from the hospital environment in Bucharest, Romania

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BACKGROUND

•Antimicrobial resistance (AMR) represent a growing public health which consist in the capacity of the microorganisms to survive exposure to antibiotic treatment . Infections caused by multidrug resistant (MDR) and virulent Gram-positive and Gram negative bacteria are very common in hospital settings but recently there have been described that are involved also in community environments.

PURPOSE AND HYPOTHESIS

•The purpose of this study was to investigate the phenotypic resistance and virulence markers in *Staphylococcus* sp., *Pseudomonas* sp, *Enterobacteriaceae* strains isolated from the hospital environment and from patients with surgical wound infections in order to obtain epidemiologically relevant data.

MATERIALS AND METHODS

•The strains identification was performed with the automated miniApi system The resistance phenotypes were established using disk diffusion and double-disk diffusion test. The isolated strains were tested for the production of different cell-associated (adherence to cellular and inert substratum) and soluble virulence factors: hemolysins, amylase, caseinase, aesculin hydrolysis, DNA-ase, lipase and lecithinase, which give microorganisms the ability to colonize and disseminate in the host. Multiplex PCR reactions were performed for the detection of the SCCmec cassette type in *S.aureus* strains, exotoxine genes *in Pseudomonas* and to identify the genetic support of cell-associated and soluble virulence factors in *Enterobacteriaceae* strains, respectively *aggA, aggR, EaaggEC,aaf1,EAST/1, hlyA, rfa and rfb* genes, which in correlation with the LPS synthesis can cause, among bacteria-induced lesions, strong inflammatory reactions, that may even lead to septic shock.

RESULTS

•In *S. aureus* isolates strains the molecular analysis showed that 60% of the isolates were MRSA and the molecular analysis revealed the presence of the SCCmec cassette type mec IVa and II types (fig. 1 and 2). *Pseudomonas* strains showed virulence genes (fig. 3 and 4). The isolated *Enterobacteriaceae* strains were resistant to beta-lactam antibiotics, including penicillins and associations with beta-lactamase inhibitors, third and fourth generation cephalosporins and carbapenems(encoded by bla_{TEM}, $bla_{NDMlike}$ fig.7*;* $bla_{CTX\text{-}Mlike}$, $bla_{OXA\text{-}48like}$ genes, fig. 6), quinolones (QnrA, gyrB, parE), aminoglycosides (aac3Ia), and tetracyclines. Most of the strains presented at least one of the seven tested virulence factors. The carbapenemases and ESBLs positive strains proved to be positive for the majority of the tested soluble virulence factors, proving the pathogenic potential of strains.

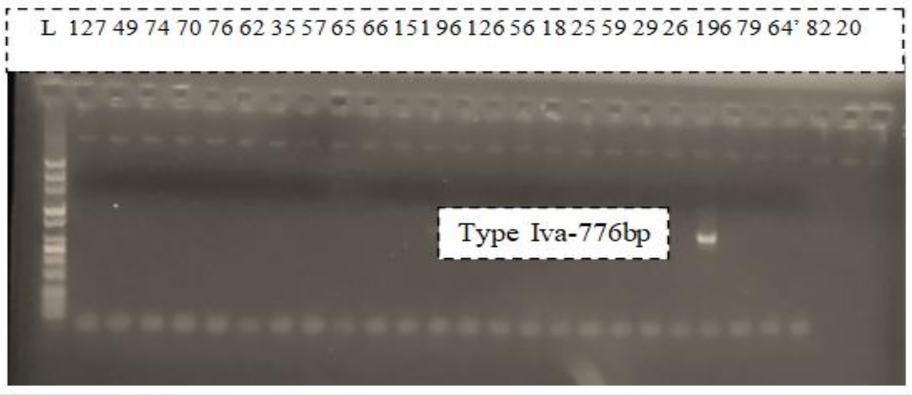

Fig. 1. Gel electrophoresis for Type IVa, Type IVb, Type IVc, Type II genes.

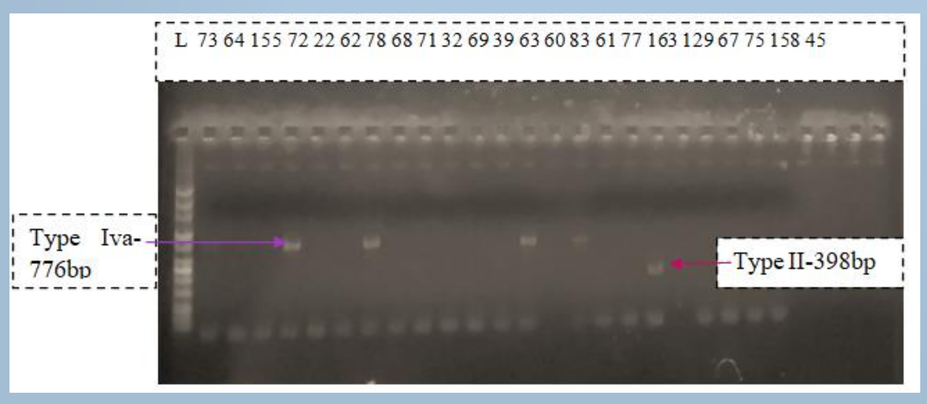

Fig. 2. Gel electrophoresis for Type IVa, Type IVb, Type IVc, Type II genes.

Figure 2. Distribution of virulence genes in analyzed strains.

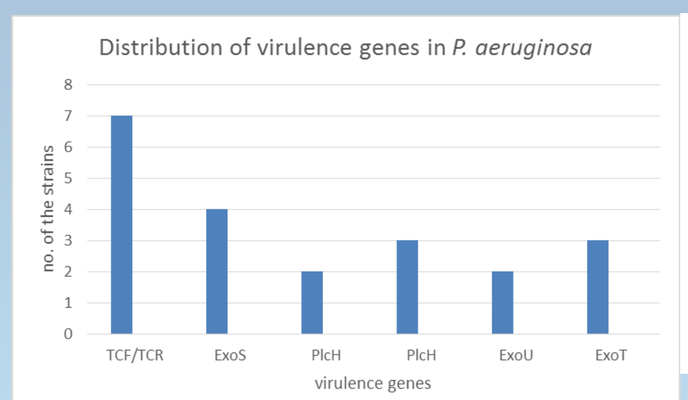

Fig.3. Distribution of the virulence genes in analyzed *P. aeruginosa* strains.

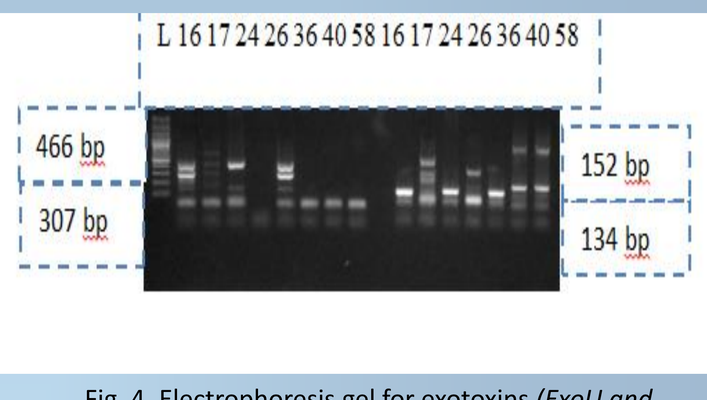

Fig. 4. Electrophoresis gel for exotoxins *(ExoU and ExoT genes*-right side*)* and *phospholipases* [*PlcH* (466pb) and*d PlcN* (307pb) genes (left side). Line 1: PCR Marker (Promega) - 100pb; positives isolates for the two phospholypases: no16, 24 and for exotoxins- ExoU-16, 24; for ExoT-24, 58 and 40.

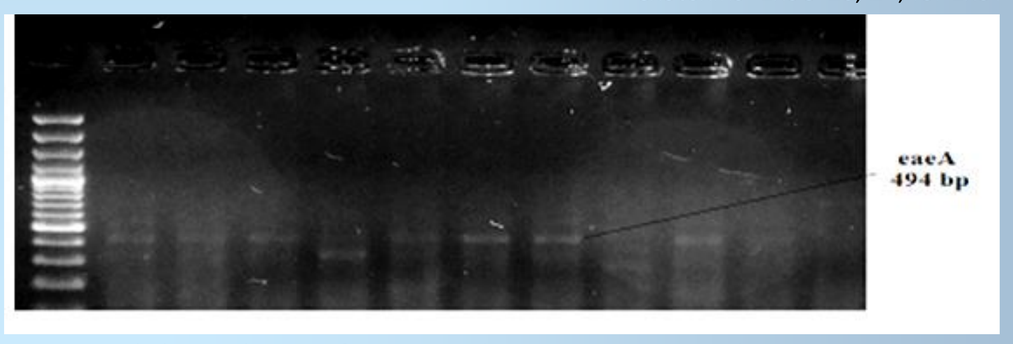

Fig. 5. Gel electrophoresis for Eaea and aggR genes in *Enterobacteriaceae* strains

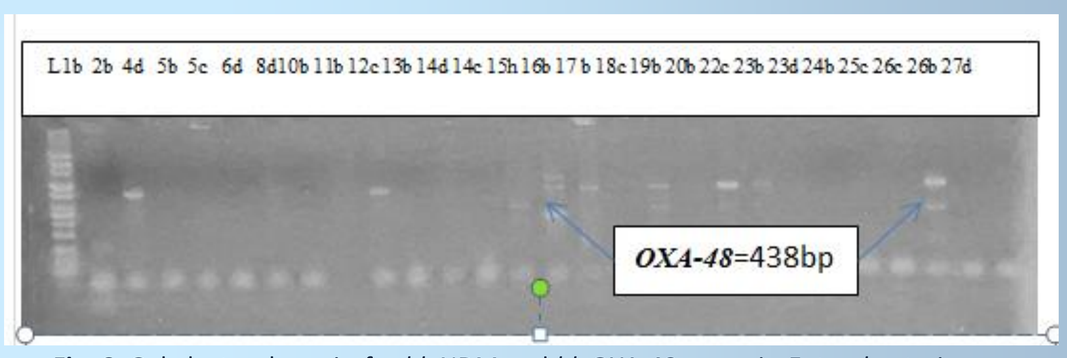

Fig. 6. Gel electrophoresis for *bla*NDM and *bla*OXA-48 genes in *Enterobacteriaceae* strains

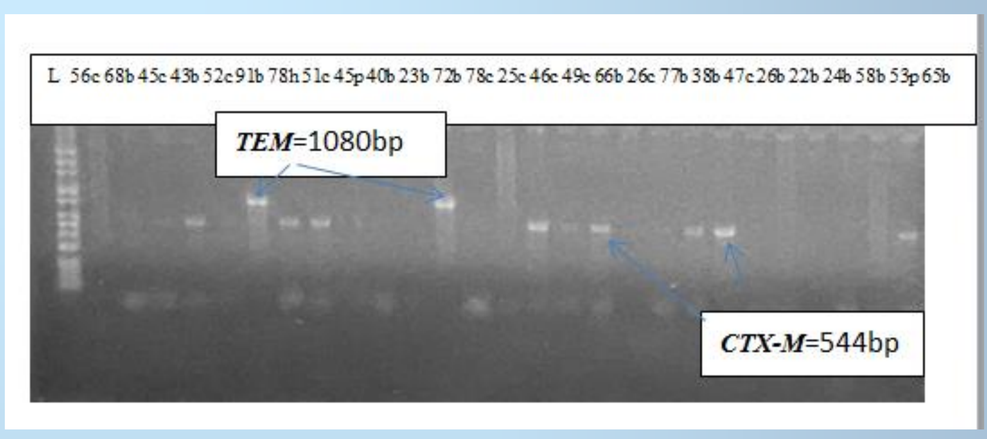

Fig. 7. Gel electrophoresis for blaCTX-M and blaTEM genes. In *Enterobacteriaceae* strains

CONCLUSIONS

•Our results showed that the isolated strains harbor multiple drug resistance and virulence determinants, suggesting the possible nosocomial origin. Resistance and virulence determinants may reside within the same plasmids and, therefore, be spread together, raising the need for the implementation of screening and intervention measures for the prevention of severe infections with virulent and resistant strains occurred in hospitalized patients.

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