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Letter to the Editor

Molecular epidemiology of *Pseudomonas aeruginosa* clinical isolates

Dear Editor,

Pseudomonas aeruginosa is an opportunistic pathogen of both community-acquired and hospital-acquired infections which rarely causes disease in healthy immunocompetent individuals. The aim of the study was to explore total plasmid contents, antibiotic susceptibility profiles and heterogeneity features of the isolates with a combined approach of Enterobacterial Repetitive Intergenic Consensus-Polymerase Chain Reaction (ERIC-PCR) and Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) profiles.

A total of 54 *P. aeruginosa* isolates were collected from various clinical specimens (urine 32%, sputum 17%, wound 15%, blood 13%, respiratory secretion 9%, aspirate 6%, abscess 2%, endotracheal aspirate 2%, pleural fluid 2%, seminal fluid 2%) of hospitalized people in Sinop and nearby cities, Turkey. Age of the hospitalized individuals ranged from 0.6 to 91 years (mean ± SD: 59.95 ± 23.09 years). Reference strain *P. aeruginosa* ATCC 27853 was used as a positive control for all physiological and biochemical tests, as well as molecular tests. All tests were performed according to Bergey's manual of systematic bacteriology.[1]

Antimicrobial susceptibility testing of 19 antibiotics was carried out according to Clinical and Laboratory Standards Institute (CLSI) recommendation. All the strains tested were resistant to sulfametoxazole/trimethoprim, tetracycline, chloramphenicol, penicillin G, and clindamycin and highly resistant to ampicillin/sulbactam, colistine, and cefotaxime. Gentamicin, imipenem, ciprofloxacin, and ceftazidime were chosen to determine multidrug resistance (MDR) and 31.4% ($n = 17$) of the isolates were defined as MDR. MDR *P. aeruginosa* rates are reported in the range of 0.6–32% according to the geographic location and surveillance study types.[2] Based on this data, the values of MDR *P. aeruginosa* in our study seem close to the highest value.

Plasmid DNA and chromosomal DNA isolation was performed according to the method of Sambrook et al.[3] with some modifications. Plasmid profiling demonstrated that all clinical isolates harbored between 3 and 6 plasmids with molecular weights ranging between 22,000 bp and 600 bp and plasmids of 14,500 bp, 1392 bp, and 983 bp were detected in all strains isolated.

ERIC-PCR and investigation of the prevalence of virulence genes *algD*, *exoS*, *lasB*, *toxA*, and *rhlAB* were performed by previously described primers and conditions[4] with some modifications. There were 3–12 bands with molecular weights ranging from 120 bp to 14,750 bp generated by ERIC primers for a total of 50 strains and reference strain. In the dendrogram, the isolates were separated into two major clusters (Z and Y) at similarity levels between 47% and 100% (Fig. 1A). The virulence genes *toxA*, *lasB*, *exoS*, *algD*, and *rhlAB* were present in 36 (66.6%), 53 (98.1%), 27 (50%), 47 (87%), and 52 (96.2%) of the isolates, respectively. So, the clinical isolates proved to harbor the virulence genes investigated at 50% or above.

Extractions of whole cell and extracellular proteins for a total of 18 clinical isolates and reference strain were carried out with the methods of Laemmli[5] and Wessel and Flügge.[6] respectively. Protein analysis was carried out with SDS-PAGE as described by Laemmli.[5] Whole cell and extracellular protein bands ranged from 200 kDa to 30 kDa and 197 kDa to 54 kDa, respectively. In the dendrograms the isolates were separated into two major clusters (A and B) between 76% and 96% (Fig. 1B) and (C and D) between 69% and 94% (Fig. 1C) for whole cell proteins and extracellular proteins, respectively.

The less discriminative power of SDS-PAGE at strain level may inevitably demand to benefit from the advantages provided by specialized molecular tools alone or in combination. ERIC-PCR is an appropriate, inexpensive, fast, reproducible and discriminatory DNA typing tool for effective epidemiological surveillance of *P. aeruginosa* isolates. Conventional antibiotics fail to effectively clear chronic infections caused by biofilms and necessitate new measures as an approaching step to post-antibiotic era. Inhibition of quorum-sensing and related virulence factors of *P. aeruginosa* thus may be a promising approach to combat with the pathogenicity and elimination of the infections.

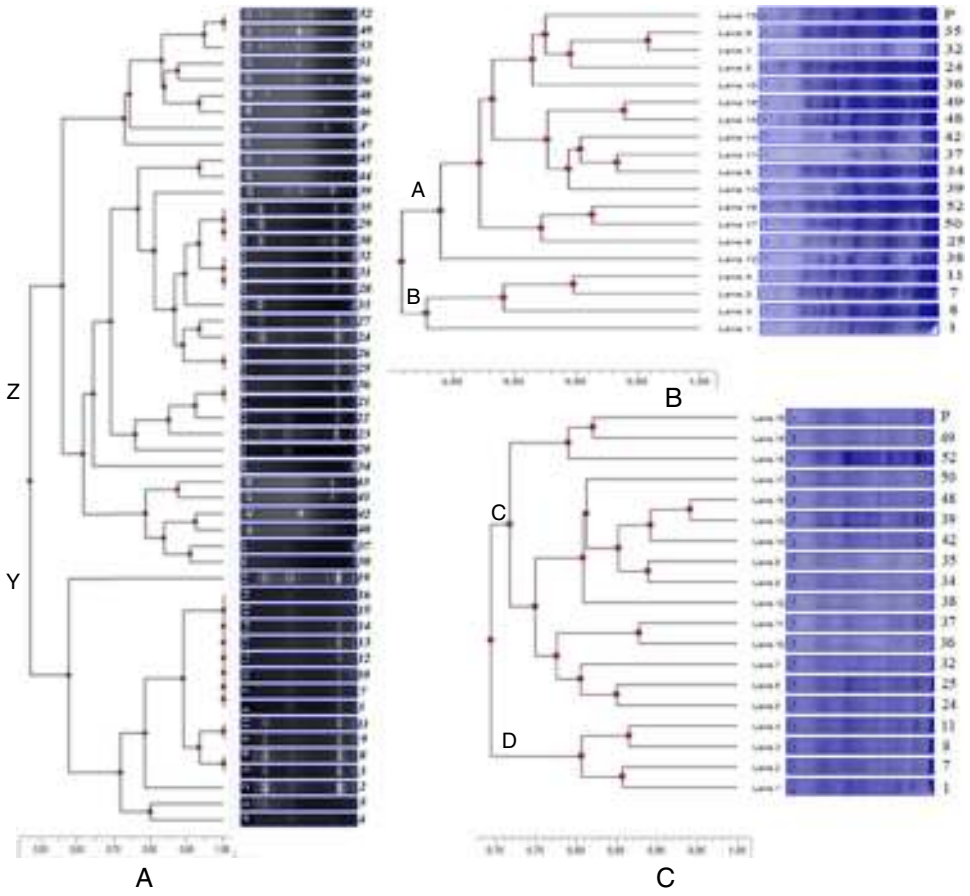

Fig. 1 – Dendrogram derived from cluster analysis (UPGMA) illustrating (A) ERIC-PCR genotype analysis, (B) SDS-PAGE analysis of whole cell proteins, and (C) SDS-PAGE analysis of extracellular proteins.

Conflicts of interest

The authors declare no conflicts of interest.

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Ismet Berber[a], Cumhur Avsar[a], Zeynep Yegin[b,*], Melike Tekerci[a], Seyhan Civek[a]

[a] *Department of Biology, Faculty of Science and Arts, Sinop University, Sinop, Turkey*

[b] *Scientific and Technological Research Application and Research Center, Sinop University, Sinop, Turkey*

[*] *Corresponding author.*
E-mail address: zyegin@sinop.edu.tr (Z. Yegin).

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