JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Silent spread of pathogens in hospital settings: a one-week molecular epidemiologic study

Elif S Tanriverdi[1], Yusuf Yakupogullari[1], Deste Ceylan[1], Yucel Duman[1], Baris Otlu[1]

[1] Department of Medical Microbiology, Inonu University Faculty of Medicine, Malatya, Türkiye

Abstract

Introduction: Molecular fingerprinting analyses of the pathogens isolated from healthcare-associated infections (HAIs) in an off-outbreak period can provide important data which cannot be obtained by prospective surveillance. Such data may indicate unnoticed breaks in infection control measures and guide in determining targeted interventions or reinforcements. The study aimed to analyze the clonal relatedness of pathogens isolated from HAIs during a period when the facility's HAI rate remained stable.

Methodology: A prospective cross-sectional study was conducted in a university hospital. A total of 105 bacterial pathogens isolated from HAIs in a one-week period were genotyped using pulsed-field gel electrophoresis.

Results: All 12 enterococci isolates belonged to one of the genotypes that infected more than one patient. There was an epidemic clone in *Staphylococcus aureus* responsible for 7 out of 12 HAIs caused by this species. Among the Gram-negative bacteria, *Acinetobacter baumannii* showed the highest clonality, with 3 genotypes responsible for 13 out of 16 HAIs caused by this species. *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* showed the lowest clonality, and their spreads involved a pair of patients in a total of 7 events.

Conclusions: This study showed that almost half of HAIs were due to clonal spread that was not detected by active surveillance. Enterococci, *S. aureus*, and *A. baumannii* had the highest clonality, suggesting that a significant proportion of HAIs could be prevented in healthcare facilities where these pathogens predominate. Repeating comprehensive studies in hospitals at regular intervals will be useful to identify unnoticed breaks in infection control measures.

Key words: infection surveillance genotyping; *Staphylococcus aureus*; *Enterococcus* spp; *Acinetobacter baumannii*.

J Infect Dev Ctries 2025; 19(7):1066-1073. doi:10.3855/jidc.20812

(Received 03 September 2024 – Accepted 10 January 2025)

Introduction

Healthcare-associated infections (HAIs) are a significant health burden around the world; and increase patient mortality and morbidity, length of hospital stay, and treatment costs [1,2]. These infections also threaten the treatment success achieved by modern medicine in the healthcare of patients with major surgery, cancer, organ failure, transplantation, critical medical conditions, and the elderly. The global increase in antimicrobial resistance (AMR) among HAI pathogens will exacerbate this burden further, and prevention practices through infection control measures (ICMs) are the only available and most rational strategy today.

Hospitalized patients are prone to develop infection because of disruption of protective anatomical barriers (due to surgery and other invasive procedures, instrumentation, and life-support applications), stress factors, organ failure, and some types of therapies that negatively affect the patients' immune systems [3,4]. Pathogens from the patients' own body flora (endogenous); or spread from an external source (exogenous) such as other patients, the physical environment, or healthcare workers, are the main causes of these infections. However, the dynamics of HAI development often involve complicated pathways and mechanisms that lead to the introduction of the pathogen into the appropriate anatomical sites of a susceptible host. Improving our understanding of the development of infections in hospitalized patients and the patterns of pathogen spread in healthcare facilities (HCFs) will help to alleviate this significant problem.

One of the substantial indicators of the overall effectiveness of ICMs is the attack rate of HAIs. When analyzing the trend, an increase in the rates of some pathogens indicates problems with patient care and/or the adequacy or compliance with ICMs. An increase in the incidence of a particular species is one of the earliest signs of a possible spread or outbreak due to ICM break. On the other hand, infections detected during periods when there is no problem with compliance with ICMs, and when the incidence of HAI is stable and below an optimal level set by health authorities and previously measured in the facility have not been adequately

detailed.

In order to increase the effectiveness of prevention studies, it is important to know how many more HAIs are preventable in a HCF where the current ICMs are applied. Characterization of these infections with a higher resolution perspective may provide additional information that will guide us to develop more targeted ICMs; and in this way, infection incidence rates in HCFs may be reduced further. However, there is very limited information on this topic.

This study aimed to determine the clonal relationship of some HAI pathogens in a tertiary-level HCF with a stable HAI incidence value, and where there was daily, prospective, and active surveillance of pathogens for about two decades.

Methodology

Study design, facility, and infection control practices

A prospective, cross-sectional molecular epidemiological study was conducted in Turgut Ozal Medical Center of Inonu University in Malatya, Türkiye. The facility is a 1600-bed tertiary level hospital with 320 intensive care unit (ICU) beds belonging to 20 different clinical departments; and one of the reference centers for organ transplantation, oncology, and trauma in the country.

The facility has an infection control (IC) team that has been implementing the current ICMs since 2003. The committee is formed with the resident physician members from infectious diseases, medical microbiology, pediatrics, chest diseases, and surgical departments; the hospital pharmacist, and the IC nurses. The IC team has been monitoring the HAI pathogens isolated daily in the facility's ICUs by active and prospective surveillance since 2005.

Pathogens and characteristics of the study period for HAI attack rates

The bacterial pathogens isolated from the inpatients of this hospital over a one-week period from 19 to 25 September 2022 were collected. In addition, the pathogens isolated from inpatient samples that were obtained after > 48 hours of patients' hospital admittances were included in the study. The samples were collected from all hospital clinics, including the ICU; and encompassed various types such as blood, urine, wound swabs, abscesses, sputum, and pleural aspirates. A total of 7 bacterial species were selected for this study, including *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumoniae*. The bacterial strains that were isolated within the study period, but their clinical samples were obtained before the study period, were excluded; and the bacterial strains that were isolated after the study period, but their clinical samples were obtained within the study period, were included.

In 2022, HAI rates in the ICUs of the facility were between 3.97 and 4.86 per 1,000 patient days; and it was 4.57 in the third quarter of the year, when this study was performed. The mean and the median HAI rates of the facility in the study month (sum of ICUs and clinics) were 9.49 and 9.2 (min–max = 4.4–14.0), respectively. These mean and median HAI rates were 9.7 and 9.9 (min–max= 7.3-12.2), respectively, in the study week. Figure 1 shows the daily HAI attack rates at the facility in September 2022.

Bacterial identification and susceptibility tests

The bacterial strains growing in the culture of the patients' clinical samples were identified with classical bacteriologic methods and with the VITEK MS matrix-assisted laser desorption ionization time of flight mass spectrometry device (MALDI TOF-MS; bio Mérieux, Durham, USA). Antimicrobial susceptibility of the isolated strains was analyzed by the disk-diffusion method, and the results were evaluated according to the guidelines of the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [5].

Genotyping with pulsed field gel electrophoresis (PFGE)

Genotyping of the isolated species was done with PFGE according to the previously published protocols [6,7].

Briefly, bacterial cells were picked from the colonies that were incubated overnight on Mueller Hinton agar (Oxoid Co, Basingstoke, UK), and suspended in cell suspension buffer (10 mM Tris-HCl, 20 mM NaCl, 50 mM EDTA). Then, the suspension was mixed with 2% low melting agarose, and plugs were prepared. Each plug was placed in the cell lysis buffer-1 (1 mg/mL lysozyme, 10 mM Tris-HCl, 50 mM NaCl) and incubated at 37 °C for 2 hours. Then the plugs were washed twice with dH_2O and placed into cell lysis buffer-2 (1 mg/mL proteinase K, 100 mM EDTA) for an overnight incubation. The plugs were washed 3 times with TE buffer and dH_2O.

Agarose plugs containing purified DNA were prepared for restriction endonuclease digestion. The restriction endonuclease enzymes (Biolabs, Beverly, USA) used were *XbaI* for *K. pneumoniae* and *E. coli*, *ApaI* for *A. baumannii*, *Spe-I* for *P. aeruginosa*, and

SmaI for *Enterococcus* and *Staphylococcus*.

Gel electrophoresis was performed using a CHEF-DR II system (Bio-Rad, Nazareth, Belgium). The gel was stained with ethidium bromide, and UV-visualized and photographed with Gel Logic 2200 (Kodak Inc., Rochester, USA). The band profiles were analyzed using the GelCompar II Software V6.6 (Applied Maths, Sint-Martens-Latem, Belgium). The Dice correlation coefficient was used to calculate similarity for band analysis, and the unweighted pair group method with arithmetic mean (UPGMA) method was used for clustering analysis.

Results

A total of 105 bacterial strains from 92 patients, of whom 55 (59.8%) were males, were analyzed. The median age of the patients was 58.5 years (min 0 – max 84); 40 patients were hospitalized in the ICU, and 52 in the clinical wards. There was growth of more than 1 bacterial species in the samples collected from 4 patients, and the same species was detected in the consecutive or different clinical samples of 9 patients.

A total of 12 *S. aureus* strains were isolated from 11 patients in 8 different wards. These isolates belonged to 6 different genotypes, of which 7 isolates of 1 genotype were isolated from 6 different patients.

A total of 6 *E. faecalis* strains were isolated from 6 patients in 5 different wards, and these strains belonged to 2 genotypes. Among these, 2 isolates from 2 different patients belonged to 1 genotype; and 4 isolates from 4 different patients belonged to a second genotype. Six *E. faecium* strains were isolated from 6 different patients

Table 1. The characteristics of patients, clinical samples, and the genotypes of the isolates studied.

Patient	Ward	Sample type	Sample Day	Genotype
***Staphylococcus aureus* (Sa)**				
A-Sa	Nephrology-I	Urine	4	**1**
B-Sa	Neurosurgery ICU	Abscess	5	**1**
B-Sa	Neurosurgery ICU	Pleural aspirate	5	**1**
C-Sa	Chest Diseases	Sputum	4	**1**
D-Sa	Nephrology ICU	Sputum	7	**1a**
E-Sa	Nephrology ICU	Wound	1	**1b**
F-Sa	Nephrology-I	Wound	7	**1b**
G-Sa	Orthopedics	Wound	4	2
H-Sa	Neurology Stroke ICU	Sputum	6	3
I-Sa	Burn ICU	Wound	1	4
J-Sa	Chest Diseases	Blood	7	5
K-Sa	Oncology-C	Wound	2	6
***Enterococcus faecalis* (Efs)**				
A-Efs	Neurology Stroke ICU	Blood	7	**1**
B-Efs[1]	Nephrology-I	Blood	2	**1**
C-Efs	Nephrology-I	Urine	6	**2**
D-Efs	Nephrology-II	Urine	4	**2**
E-Efs	Chest Diseases ICU	Sputum	1	**2**
F-Efs[2]	Burn ICU	Blood	7	**2**
***Enterococcus faecium* (Efm)**				
A-Efm	Nephrology ICU	Urine	3	**1**
B-Efm	Nephrology-I	Urine	6	**1**
C-Efm	General Surgery ICU	Urine	7	**2**
D-Efm	Toxicology ICU	Blood	1	**2**
E-Efm	Chest Diseases	Urine	6	**2**
F-Efm	General Surgery	Urine	2	**2a**
***Pseudomonas aeruginosa* (Pa)**				
A-Pa	Liver Tx-a/II	Blood	4	**1**
A-Pa	Liver Tx a/II	Blood	5	**1**
B-Pa	Reanimation-II	Urine	7	**1**
C-Pa	General Surgery ICU	Wound	3	**2**
C-Pa	General Surgery ICU	Sputum	5	**2**
D-Pa	Oncology-C	Sputum	2	**3**
D-Pa	Oncology-C	Sputum	4	**3**
E-Pa	Oncology-C	Sputum	4	**3a**
F-Pa	Neurology Stroke ICU	Urine	6	**4**
F-Pa	Neurology Stroke ICU	Urine	5	**4**
G-Pa[3]	Oncology-C	Sputum	5	5
H-Pa	Orthopedics	Wound	6	6
I-Pa	Burn ICU	Wound	3	7
J-Pa[4]	Liver Tx-c/I	Urine	4	8
K-Pa	Orthopedics	Urine	5	9
L-Pa	Oncology-C	Sputum	6	10
M-Pa	Liver Tx-c/I	Wound	4	11

Table 1 (continued). The characteristics of patients, clinical samples, and the genotypes of the isolates studied.

Patient	Ward	Sample type	Sample Day	Genotype
***Escherichia coli* (Ec)**				
A-Ec	Hematology	Urine	6	1
B-Ec	Gastroenterology-I	Sputum	5	2
C-Ec	Cardiovascular Surgery	Urine	6	3
D-Ec[1]	Nephrology-I	Blood	2	4
E-Ec	Endocrinology	Urine	3	5
F-Ec	Nephrology-I	Urine	7	6
G-Ec	Neurosurgery	Peritoneal aspirate	7	7
H-Ec	General Surgery	Sputum	4	8
I-Ec	Bone Marrow Tx	Urine	2	9
J-Ec	Reanimation ICU-II	Sputum	6	10
K-Ec	Burn ICU	Wound	1	11
L-Ec[3]	Oncology-C	Sputum	5	12
M-Ec	Liver Tx-b/II	Blood	1	**13**
N-Ec	Oncology ICU	Wound	7	**13**
O-Ec	Gastroenterology-I	Urine	3	14
P-Ec	Nephrology-I	Urine	6	15
Q-Ec	Pediatric Hematology	Urine	6	16
R-Ec	Hematology	Blood	3	**17**
S-Ec	Hematology ICU	Urine	4	**17a**
T-Ec	Chest Diseases ICU	Urine	5	**18**
T-Ec	Chest Diseases ICU	Sputum	6	**18**
U-Ec	General Surgery	Abscess	6	19
V-Ec	Neurosurgery ICU	Urine	2	**20**
V-Ec	Neurosurgery ICU	Urine	3	**20**
W-Ec	Gastroenterology-I	Blood	7	21
X-Ec[4]	Liver Tx-c/I	Urine	4	**22**
Y-Ec	Liver Tx-b/ICU	Blood	5	**22**
Z-Ec	Gastroenterology ICU	Urine	5	23
***Klebsiella pneumoniae* (Kp)**				
A-Kp	Nephrology-I	Urine	2	**1**
B-Kp	Nephrology ICU	Blood	1	**1**
C-Kp	Chest Diseases	Sputum	4	2
D-Kp	Nephrology ICU	Urine	3	3
E-Kp	Pediatrics ICU	Blood	6	4
F-Kp	Orthopedics	Sputum	7	5
G-Kp	Gastroenterology-P	Urine	1	6
H-Kp	Neurosurgery	Urine	1	7
I-Kp	Chest Diseases ICU	Sputum	3	8
J-Kp	Reanimation ICU-I	Sputum	3	9
K-Kp	Gastroenterology ICU	Urine	5	10
L-Kp	Liver Tx-a/II	Blood	2	**11**
M-Kp	Liver Tx-b/I	Blood	1	**11a**
M-Kp	Liver Tx-b/I	Blood	5	**11a**
N-Kp	Hematology	Urine	3	12
O-Kp	Liver Tx-O/II	Peritoneal Aspirate	7	13
P-Kp	Gastroenterology	Sputum	2	14
Q-Kp	Cardiology	Urine	4	15
R-Kp	Neurology	Urine	7	16
S-Kp	Oncology-A	Sputum	5	17
***Acinetobacter baumannii* (Ab)**				
A-Ab	Oncology-C	Wound	1	**1**
B-Ab	Neurosurgery ICU	Sputum	3	**1**
C-Ab	Chest Diseases ICU	Urine	6	**1**
D-Ab	Neurology Stroke ICU	Sputum	6	**1**
E-Ab	Chest Diseases ICU	Sputum	2	**1**
E-Ab	Chest Diseases ICU	Wound	5	**1**
F-Ab	Reanimation ICU-I	Sputum	6	**1**
G-Ab	Neurosurgery ICU	Sputum	7	**1a**
H-Ab	Hematology ICU	Urine	1	**1b**
I-Ab	Neurology Stroke ICU	Sputum	1	2
J-Ab[2]	Burn ICU	Blood	7	**3**
K-Ab	Reanimation ICU-I	Sputum	6	**3a**
L-Ab	Cardiology	Urine	1	4
M-Ab	Orthopedics	Sputum	5	5
N-Ab	Reanimation ICU-II	Sputum	4	**6**
O-Ab	Reanimation ICU-I	Sputum	5	**6b**

ICU: intensive care unit; Tx: transplantation. *The patients who were infected by more than one species were marked with superscript numbers. Bold font indicates genotypes shared by more than one patient isolate.

in 6 different wards, and they belonged to 2 genotypes. Among these, 2 isolates belonged to 1 genotype, and 4 isolates belonged to the second genotype.

A total of 17 *P. aeruginosa* strains were isolated from 13 patients in 8 different wards. The strains belonged to 11 genotypes. Among these, 3 isolates from 2 patients in 2 different wards were of genotype 1, and 3 isolates from 2 different patients in the same ward belonged to genotype 3.

A total of 28 *E. coli* strains were isolated from 26 patients in 20 different wards. The strains belonged to 23 different genotypes. There were 5 genotypes (genotypes 13, 17, 18, 20, and 22) that were isolated more than once and consisted of 2 isolates each. Among them, genotypes 13, 17, and 22 were isolated from different patients.

Twenty *K. pneumoniae* were isolated from 19 patients in 18 different wards. The isolates belonged to 17 genotypes. Genotype-1 included 2 strains from 2 different patients in different wards; and genotype-11 included 3 isolates from 2 patients in 2 different wards.

A total of 16 *A. baumanni* strains were isolated from 15 patients in 10 different clinical wards. They belonged to 6 genotypes, of which the genotype-1 included 9 isolates from 8 patients from 6 different wards. There were 2 isolates each of genotype-3 and -6, from different patients and wards.

Table 1 summarizes the patients' clinics, clinical samples, and the genotype characteristics of the isolates analyzed in this study. Supplementary Figure 1 presents a dendrogram analysis of the studied strains.

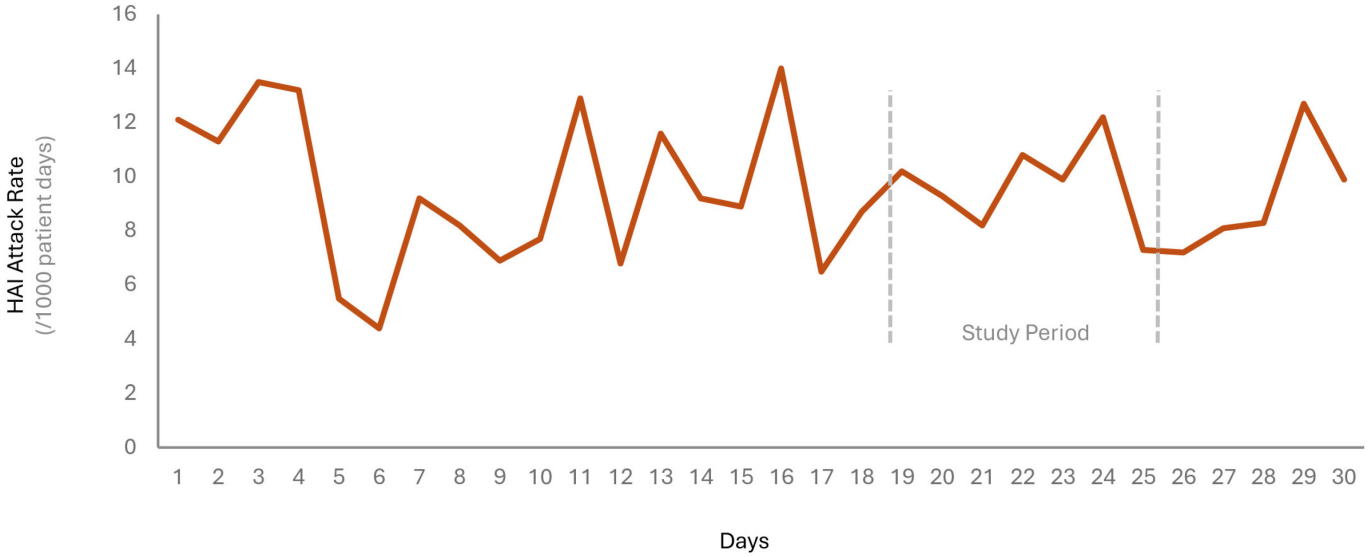
Discussion

Our study revealed significant insights into the clonal relationships of HAI pathogens during a period of stable HAI incidence. The molecular fingerprinting analysis identified previously unnoticed transmission patterns, indicating potential breaches in infection control practices that were not detectable through conventional surveillance methods. These findings emphasize the importance of molecular surveillance even in periods with low or stable HAI rates, as they can unveil hidden risks and inform targeted intervention.

We carried out this study in a hospital with a stable HAI incidence for at least a decade and a lower level of HAI attack rates compared with similar HCFs in our country [8]. It was also performed during a period when the hospital's IC team did not conduct any outbreak investigation, and there were no significant differences in HAI attack rates between the study week and the other weeks of the month (Figure 1). Thus, in this study, we were able to analyze HAI data that were not indicating a considerable clonal expansion when viewed from a classical surveillance perspective.

We found that all 7 bacterial species spread clonally among hospitalized patients in the facility; and Gram-positive species, including enterococci and *S. aureus*, had the highest clustering rates. There was no unique genotype among the enterococci, and each isolate belonged to a genetic clone that infected more than 1 patient. This indicated that HAIs caused by enterococcus species in our hospital developed as a result of the pathogens circulating in the facility and transmitted to patients, and that there might be

Figure 1. The daily healthcare-associated infection (HAI) attack rates at the facility in September 2022.



consistent deficiencies in our infection control practices for preventing the spread of pathogens. Eleven of the 12 enterococcal strains were isolated from urine or blood, and 10 of these patients had urinary and/or vascular catheters. Therefore, we suggest beginning with a focus on catheter insertion and maintenance practices, as virulent strains can more effectively colonize inanimate surfaces.

On the other hand, *S. aureus* showed a different genotype distribution pattern in HAIs than enterococci. There was an epidemic *S. aureus* clone that infected 6 patients, and the remaining ones were all unique. The spread of the predominant clone (most likely a hypervirulent clone) occurred in the wards that were close to each other. Furthermore, clonally related enterococci and *S. aureus* were isolated mostly from some of the wards; and this has also suggested possible problems with hand hygiene and effective cleaning of the toilets in these areas, in addition to the other possible reasons mentioned above.

In the last two decades, there has been a significant increase in the frequency of Gram-negative bacteria among HAI pathogens [9]. This is due to the effective escalation of these organisms from antibiotic pressure because of their ability to develop resistance to the last line drugs [10]. Despite their high proportion (about 77%) among all HAI pathogens found in this study, there was less clonality among Gram-negative species compared to Gram-positive species. In particular, *K. pneumoniae* and *E. coli* had the lowest clonality, suggesting that these two pathogens were not clonally expanding at a significant level in our facility, but rather were the cause of endogenous HAIs. Therefore, these results may also indicate the need to reconsider our patient care processes to reduce the development of endogenous infections. In addition, finding a low clonality for *P. aeruginosa*, an important hospital pathogen with high potential for epidemic spread in HCFs, was an interesting finding of this study. Nevertheless, there were limited spreads of these 3 Gram-negative species, each involving 2 patients. These spreads were mostly within the clinic, but 1 *E. coli* (genotype 13) and 1 *P. aeruginosa* (genotype 1) spread between patients staying in satellite hospitals, such as the liver transplantation (Tx) institute and the oncology hospital, connected by tube-passes to the main hospital building. One of these facilities (the liver Tx institute) had its own operating theatres and radiology department, and both facilities had their own ICUs; therefore, it was reasonable to predict that these spread events were most likely caused by healthcare workers visiting the patients; or by other hospital staff distributing meals, medications, or bed linen; who circulated between the main hospital and these two buildings.

Among the Gram-negative species, the highest clonality was observed for *A. baumannii*. The frequency of this species has increased significantly since the early 2000s and it has become one of the predominant HAI pathogens in several countries, because of its ability to develop resistance efficiently to many classes of drugs [11,12]. This bacterium is also able to survive for long periods in the physical environment of hospitals and is relatively resistant to standard cleaning and disinfection measures [13,14]. Our study also showed that this species could cause a significant number of infections through clonal expansion. We found a total of 3 epidemic clones of *A. baumannii*, and that 1 of them (genotype 1) had spread among 8 patients in several ICUs located in both the main building and one of the satellite hospitals. The results of this study, together with previously reported data, have highlighted the need for a global collaboration against *A. baumannii*.

In this study, we used PFGE for genotyping of the strains. PFGE is a reference method with high reproducibility and discriminatory power in molecular fingerprinting of a wide variety of HAI pathogens, including the species studied here [15,16]. We observed that this method was highly consistent in the genotyping of the consecutive strains isolated on different days in a total of 6 patients, and in the genotyping of 3 strains isolated from different sample types in 3 patients.

This study was conducted over a period of 1 week. Although this was one of the limitations of our study, it did show 4 clonal spreads of some specific HAI pathogens involving 4 to 8 patients within a relatively short time; and 9 small spreads for almost all bacterial species, each involving a pair of patients. The pathogens that caused clonal spread involving relatively more patients were *S. aureus* and *Enterococcus* spp.; but they had the lowest attack rates among all HAI pathogens. *A. baumannii* was one of the predominant pathogens in our hospital for years, especially in ICU patients who had previously received wide spectrum antimicrobial therapies. Therefore, we believe that the clonal spread of these 3 pathogens did not receive sufficient attention from the IC team during the evaluation of daily surveillance results. Regarding the small spreads found in this study, we thought that these spreads were most likely the result of intermittent and low intensity ICM breaks in several clinics, and they could be detected solely by molecular epidemiological analyses. Therefore, a molecular epidemiologic study

over a longer period of time would show the exact size of these small clonal spreads.

We believe that every hospital should perform such molecular fingerprinting studies to understand how large and widespread the problem identified by this study at our institution is, in other HCFs. Repeating such studies at regular intervals will also provide important information on when, how, and by whom these epidemic clones enter the circulation, and how their spread ends. Such analyses should include sampling of staff and the environment as well, and investigation of the virulence factors of the epidemic clones. This will help to understand which factor is most likely to be associated with clonal spread, why a particular clone survived longer in the hospital environment, and why ICMs are not as effective for the epidemic genotype as they are for other genotypes in the species. In particular, the combination of virulence data with the multi-locus sequence types of the clones with a high potential for spread will be very helpful for infection control professionals to globalize the local data obtained from each HCF.

We carried out this study on the basis of laboratory data only, without using the patients' clinical information. This is the main limitation of our study, as we could not assess the exact dynamics of the spreads determined in this study. Therefore, future studies must be strengthened by using data on the clinical background of the patients to identify the "hot points" that facilitated the transfer of pathogens from one patient to another.

Increasing AMR among Gram-negative HAI pathogens to the last-line drugs has been a growing problem in our country for more than two decades [17,18]. This problem results in a vicious cycle that requires the use of more potent antibiotics in the treatments of HAIs, and this ultimately fuels the increase in AMR. Therefore, analyzing the developmental dynamics of each HAI by molecular epidemiologic tools and initiating effective studies to identify the weak points will also contribute to solving the problem of high AMR, which is becoming a global crisis.

Conclusions

In this study, we found that approximately half of HAIs in a university hospital were due to unnoticed spread, suggesting that such a considerable proportion of infection can be prevented. As we move into an era where the antimicrobials will likely fail and prevention measures will be much more important, understanding the answers to the question "how many more of HAIs are preventable" will provide important insights and opportunities to reduce HAIs. By understanding the developmental dynamics of HAIs and the spread characteristics of each HAI pathogen, we will get closer to the goal of minimum HAIs.

Corresponding author

Elif Seren Tanriverdi, MD.
Department of Medical Microbiology, Inonu University Faculty of Medicine,
Bulgurlu Mahallesi, Elazığ Yolu 15. Km., 44280 Battalgazi, Malatya, Türkiye.
Tel: +90 (422) 341 06 60
Fax: +90 (422) 341 07 29
Email: seren.tanriverdi@inonu.edu.tr

Conflict of interests

No conflict of interests is declared.

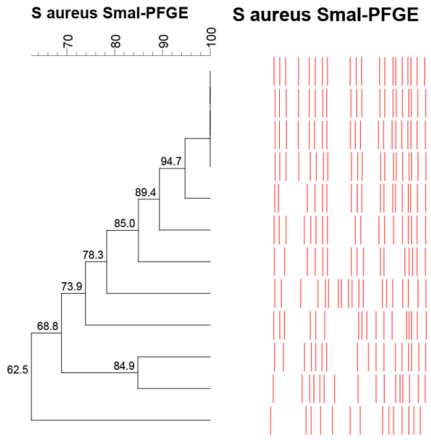
References

1. Manoukian S, Stewart S, Graves N, Mason H, Robertson C, Kennedy S, Pan J, Kavanagh K, Haahr L, Adil M, Dancer SJ, Cook B, Reilly J (2021) Bed-days and costs associated with the inpatient burden of healthcare-associated infection in the UK. J Hosp Infect 114: 43–50. doi: 10.1016/j.jhin.2020.12.027.
2. World Health Organization (2022) Global report on infection prevention and control. Available: https://iris.who.int/bitstream/handle/10665/354489/9789240051164-eng.pdf. Accessed: 4 April 2025.
3. Stewart S, Robertson C, Kennedy S, Kavanagh K, Haahr L, Manoukian S, Mason H, Dancer S, Cook B, Reilly J (2021) Personalized infection prevention and control: identifying patients at risk of healthcare-associated infection. J Hosp Infect 114: 32–42. doi: 10.1016/j.jhin.2021.03.032.
4. Rodríguez-Acelas AL, de Abreu Almeida M, Engelman B, Cañon-Montañez W (2017) Risk factors for health care-associated infection in hospitalized adults: systematic review and meta-analysis. Am J Infect Control 45: e149–156. doi: 10.1016/j.ajic.2017.08.016.
5. European Committee on Antimicrobial Susceptibility Testing, EUCAST (2022) Breakpoint tables for interpretation of MICs and zone diameters v12.0. Available: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_12.0_Breakpoint_Tables.pdf. Accessed: 4 April 2025.
6. Durmaz R, Otlu B, Koksal F, Hosoglu S, Ozturk R, Ersoy Y, Aktas E, Gursoy NC, Caliskan A (2009) The optimization of a rapid pulsed-field gel electrophoresis protocol for the typing of *Acinetobacter baumannii*, *Escherichia coli* and *Klebsiella* spp. Jpn J Infect Dis 62: 372–377. doi: 10.7883/yoken.JJID.2009.372.
7. Golding GR, Campbell J, Spreitzer D, Chui L (2015). Pulsed-field gel electrophoresis of *Staphylococcus aureus*. Methods Mol Biol 1301: 85–93. doi: 10.1007/978-1-4939-2599-5_8.
8. Gozel MG, Hekimoglu CH, Gozel EY, Batir E, McLaws ML, Mese EA (2021) National Infection Control Program in Turkey: the healthcare associated infection rate experiences over 10 years. Am J Infect Control 49: 885–892. doi: 10.1016/j.ajic.2020.12.013.

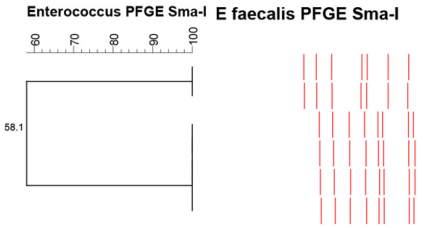
9. Aliyu S, Smaldone A, Larson E (2017) Prevalence of multidrug-resistant Gram-negative bacteria among nursing home residents: a systematic review and meta-analysis. Am J Infect Control 45: 512–518. doi: 10.1016/j.ajic.2017.01.022.
10. Peleg AY, Hooper DC (2010) Hospital-acquired infections due to Gram-negative bacteria. N Engl J Med 362: 1804–1813. doi: 10.1056/NEJMra0904124.
11. Yetkin F, Yakupogullari Y, Kuzucu C, Ersoy Y, Otlu B, Colak C, Parmaksız N (2018) Pathogens of intensive care unit-acquired infections and their antimicrobial resistance: a 9-year analysis of data from a university hospital. Jundishapur J Microbiol 11: e67716. doi: 10.5812/jjm.67716.
12. Kim YA, Park YS, Youk T, Lee H, Lee K (2018) Abrupt increase in rate of imipenem resistance in *Acinetobacter baumannii* complex strains isolated from general hospitals in Korea and correlation with carbapenem administration during 2002–2013. Ann Lab Med 38: 179–181. doi: 10.3343/alm.2018.38.2.179.
13. Yakupogullari Y, Otlu B, Ersoy Y, Kuzucu C, Bayindir Y, Kayabas U, Togal T, Kizilkaya C (2016) Is airborne transmission of *Acinetobacter baumannii* possible: a prospective molecular epidemiologic study in a tertiary care hospital. Am J Infect Control 44: 1595–1599. doi: 10.1016/j.ajic.2016.05.022.
14. Fahy S, O'Connor JA, Lucey B, Sleator RD (2023) Hospital reservoirs of multidrug resistant *Acinetobacter* species—the elephant in the room! Br J Biomed Sci 80: 11098. doi: 10.3389/bjbs.2023.11098.
15. Neoh HM, Tan XE, Sapri HF, Tan TL (2019) Pulsed-field gel electrophoresis (PFGE): a review of the "gold standard" for bacteria typing and current alternatives. Infect Genet Evol 74: 103935. doi: 10.1016/j.meegid.2019.103935.
16. Wolska K andSzweda P (2012) Genotyping techniques for determining the diversity of microorganisms. In Caliskan M, editor. Genetic Diversity in Microorganisms. InTech 2012: 53–94. doi: 10.5772/35101.
17. Poirel L, Yakupoğullari Y, Kizirgil A, Dogukan M, Nordmann P (2009) VIM-5 metallo-beta-lactamase-producing *Pseudomonas putida* from Turkey. Int J Antimicrob Agents 33: 287. doi: 10.1016/j.ijantimicag.2008.07.018.
18. Yakupogullari Y, Otlu B, Dogukan M, Gursoy C, Korkmaz E, Kizirgil A, Ozden M, Durmaz R (2008) Investigation of a nosocomial outbreak by alginate-producing pan-antibiotic-resistant *Pseudomonas aeruginosa*. Am J Infect Control 36: e13–18. doi: 10.1016/j.ajic.2008.07.006.

Annex – Supplementary Items

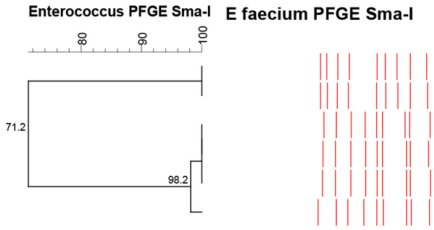
Supplementary Figure 1. Dendrogram analysis of strains.



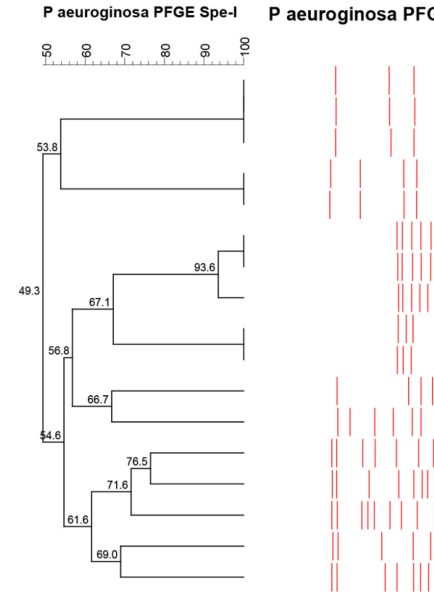
Isolate No	Genotype	Unit
31	1	Nephrology-I
38	1	Neurosurgery ICU
39	1	Chest Diseases
59	1	Neurosurgery ICU
97	1a	Nephrology ICU
5	1b	Nephrology ICU
98	1b	Nephrology-I
48	2	Orthopedics
69	3	Neurology Stroke ICU
14	4	Burn ICU
108	5	Chest Diseases
1	6	Oncology-C



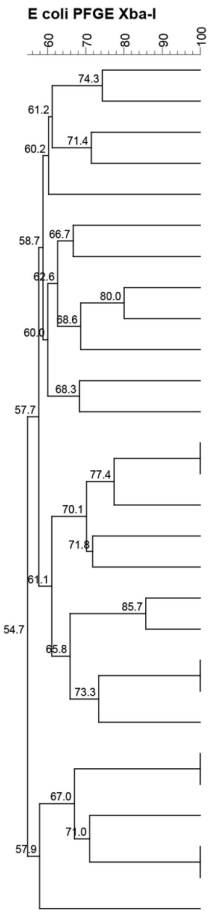
Isolate No	Genotype	Unit
106	1	Neurology stroke ICU
109	1	Nephrology-I
26	2	Nephrology-I
57	2	Nephrology-II
64	2	Chest diseases ICU
102	2	Burn ICU

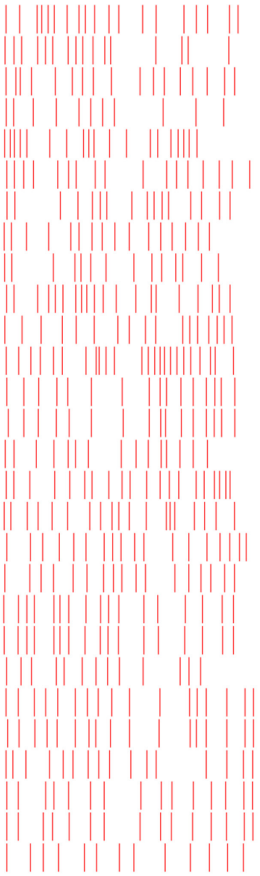


Isolate No	Genotype	Unit
76	1	Nephrology ICU
61	1	Nephrology-I
96	2	General surgery ICU
104	2	Toxicology ICU
119	2	Chest diseases
120	2a	General surgery

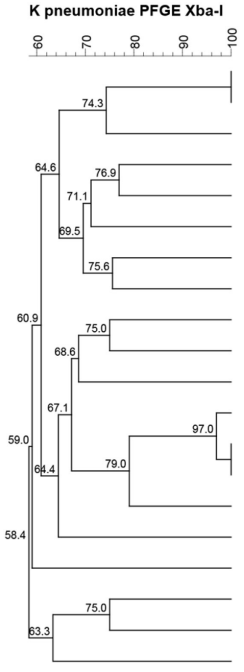


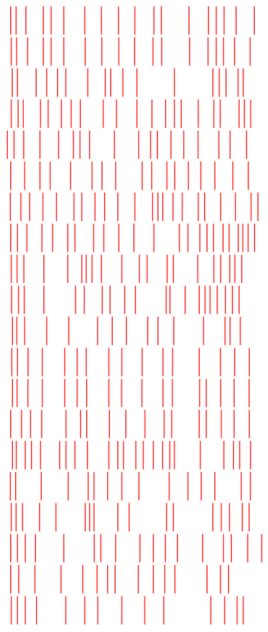
Isolate No	Genotype	Unit
107	1	Liver Tx-a/II
99	1	Liver Tx-a/II
88	1	Reanimation-II
52	2	General surgery ICU
52	2	General surgery ICU
89	3	Oncology-C
89	3	Oncology-C
72	3a	Oncology-C
87	4	Neurology stroke ICU
90	4	Neurology stroke ICU
91b	5	Oncology-C
111	6	Orthopedics
46	7	Burn ICU
47	8	Liver Tx-c/I
86	9	Orthopedics
74	10	Oncology-C
29b	11	Liver Tx-c/I



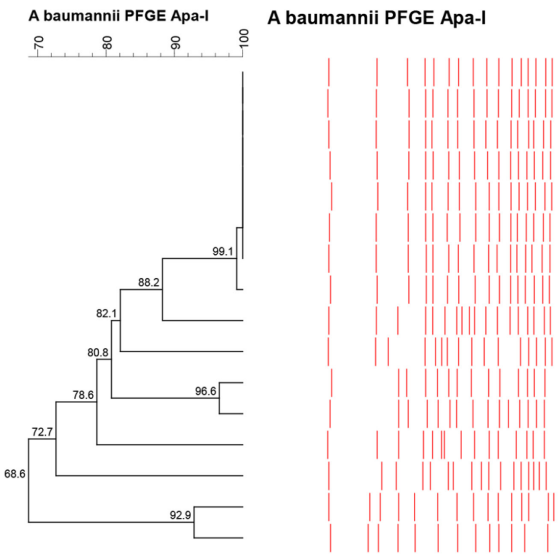


Isolate No	Genotype	Unit
58	1	Hematology
68	2	Gastroenterology-I
67	3	Cardiovascular surgery
109a	4	Nephrology-I
81	5	Endocrinology
37	6	Nephrology-I
85	7	Neurosurgery
80	8	General surgery
82	9	Bone marrow Tx
84	10	Reanimation ICU-II
118	11	Burn ICU
91a	12	Oncology-C
114	13	Liver Tx-b/II
116	13	Oncology ICU
117	14	Gastroenterology-I
66	15	Nephrology-I
79	16	Pediatric hematology
110	17	Hematology
24	17a	Hematology ICU
35	18	Chest diseases ICU
32	18	Chest diseases ICU
56	19	General surgery
62	20	Neurosurgery ICU
13	20	Neurosurgery ICU
4	21	Gastroenterology-I
121	22	Liver Tx-c/I
29a	22	Liver Tx-b/ICU
33	23	Gastrorenterology ICU





Isolate No	Genotype	Unit
8	1	Nephrology-I
18	1	Nephrology ICU
54	2	Chest diseases
20	3	Nephrology ICU
112	4	Pediatrics ICU
93	5	Orthopedics
9	6	Gastroenterology-P
19	7	Neurosurgery
22	8	Chest diseases ICU
25	9	Reanimation ICU-I
78	10	Gastroenterology ICU
100	11	Liver Tx-a/II
101	11a	Liver Tx-b/I
119	11a	Liver Tx-b/I
34	12	Hematology
7	13	Liver Tx-O/II
6	14	Gastroenterology
65	15	Cardiology
92	16	Neurology
60	17	Oncology-A



Isolate No	Genotype	Unit
15	1	Oncology-C
21	1	Neurosurgery ICU
44	1	Chest diseases ICU
94	1	Neurology stroke ICU
11	1	Chest diseases ICU
53	1	Chest diseases ICU
63	1	Reanimation ICU-I
45	1a	Neurosurgery ICU
42	1b	Hematology ICU
12	2	Neurology stroke ICU
102	3	Burn ICU
95	3a	Reanimation ICU-I
16	4	Cardiology
43	5	Orthopedics
41	6	Reanimation ICU-II
40	6b	Reanimation ICU-I

ICU: intensive care unit; PFGE: pulsed-field gel electrophoresis; Tx: transplantation.