Results of antimicrobial resistance surveillance in Uzbekistan, 2019–2022

World Health Organization
European Region

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ABSTRACT

The Proof-of-principle routine diagnostic project, conducted in Uzbekistan from 2019 to 2022, focused on enhancing antimicrobial resistance (AMR) surveillance by improving microbiological diagnostics for bloodstream infections. Implemented across four health-care facilities and the National AMR Center, the project aimed to establish standardized BC sampling, strengthen antimicrobial susceptibility testing (AST), and strengthen collaboration between clinical and laboratory teams. The study enrolled 2063 participants, identifying 299 positive cultures with significant pathogens such as *Staphylococcus aureus, Escherichia coli and Klebsiella pneumoniae*. Resistance rates were high, with notable multidrug resistance in *K. pneumoniae* and methicillin resistance in *S. aureus*. The initiative successfully improved clinical workflows, trained health-care professionals, and contributed to the accreditation of the National AMR Center. Challenges included delays in AST turnaround times, procurement issues and adherence to sampling protocols. Recommended actions emphasize the need for expanding microbiological capacity, improving diagnostic timeliness, and sustaining national AMR surveillance to guide effective treatment policies and antimicrobial stewardship.

KEYWORDS

DRUG RESISTANCE, MICROBIAL; BLOODSTREAM INFECTION; MICROBIAL SENSITIVITY TESTS; PUBLIC HEALTH SURVEILLANCE; ANTIMICROBIAL STEWARDSHIP; DRUG RESISTANCE, MULTIPLE

Document number: WHO/EURO:2025-12193-51965-79699 (PDF)

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ACKNOWLEDGEMENTS

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The project was initiated by the WHO Regional Office for Europe, in collaboration with the Netherlands National Institute for Public Health and the Environment and the European Society of Clinical Microbiology and Infectious Diseases. The support of national institutions, particularly the Ministry of Health of the Republic of Uzbekistan, was instrumental in carrying out this project.

Katherine Kooij (previously WHO Collaborating Centre for AMR Epidemiology and Surveillance, Netherlands National Institute for Public Health and the Environment) and Saskia Nahrgang (previously WHO Regional Office for Europe) are acknowledged for their role in project initiation.

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The WHO Regional Office for Europe would also like to gratefully acknowledge the lead microbiologist and clinicians from the four project sites: Zukhra Ulimbekova and Gulnoza Kurbanova (City Clinical Hospital No. 1); Munira Rasulmukhamedova and Akmal Bobodjonov (City Clinical Infectious Diseases Hospital No. 1); Shaira Okhundjanova and Manzura Baltabayeva (Republican Specialized Scientific Practice Medical Centre of Paediatrics); and Amir Bektimirov and Nigora Tadjiyeva (Republican Centre), as well as the three laboratories that participated in the project: City Clinical Infectious Diseases Hospital; City Clinical Hospital; Republican Specialized Scientific Practice Medical Centre of Paediatrics.

Thanks are also extended to the National AMR Centre under the Research Institute of Epidemiology, Microbiology and Infectious Diseases of the Ministry of Health.

ABBREVIATIONS

AMR	antimicrobial resistance
AST	antimicrobial susceptibility test(ing)
A. baumannii	*Acinetobacter baumannii*
AWaRE Access	Access, Watch and Reserve classification of antibiotics
BC	blood culture
BSI	bloodstream infection
CAESAR	Central Asian and European Surveillance of Antimicrobial Resistance
CLSI	Clinical and Laboratory Standards Institute
E. coli	*Escherichia coli*
E. faecalis	*Enterococcus faecalis*
E. faecium	*Enterococcus faecium*
EUCAST	European Committee on Antimicrobial Susceptibility Testing
HCF	health-care facility
K. pneumoniae	*Klebsiella pneumoniae*
N. meningitidis	*Neisseria meningitidis*
P. aeruginosa	*Pseudomonas aeruginosa*
PoP project	Proof-of-principle antimicrobial resistance routine diagnostics surveillance project
PoP project in Uzbekistan	Proof-of-principle routine diagnostic project for antimicrobial resistance surveillance in Uzbekistan
SIRS	Systemic Inflammatory Response Syndrome
spp.	species (for specific bacteria)
S. aureus	*Staphylococcus aureus*
S. pneumoniae	*Streptococcus pneumoniae*

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EXECUTIVE SUMMARY

Bloodstream infections (BSIs) are severe and often life-threatening infections caused by bacteria or other organisms entering the bloodstream. Effective management relies on routine microbiological examinations to isolate and identify the causative agent, enabling antimicrobial susceptibility testing (AST) for targeted therapy. Underuse of microbiological diagnostics, combined with the need to initiate empiric antimicrobial therapy, compromises patient care quality and may contribute to the emergence and spread of antimicrobial resistance (AMR). In Uzbekistan, AMR represents a significant public health concern, with a substantial impact on health outcomes. The Proof-of-principle routine diagnostic project for antimicrobial resistance surveillance in Uzbekistan aimed to identify gaps in BSI diagnostics in Uzbekistan and promote the use of laboratory testing to inform prescribing practices. Conducted across four health-care institutions and the National AMR Centre, the project focused on introducing routine microbiological diagnostics for suspected BSI cases. Data were collected on BC and AST results, demographics, clinical diagnoses and recent antimicrobial therapy.

A total of 2063 participants were enrolled in the project, with the largest group being paediatric patients. Forty-three per cent had received antimicrobial therapy in the 3 months before sample collection, and 62% were on therapy during the collection of blood samples. Positive BC were confirmed in 299 patients, with 305 isolated microorganisms. Most isolates were *Neisseria meningitidis* ($n = 101$), followed by *Staphylococcus aureus* ($n = 33$), *Enterococcus* spp. ($n = 25$), *Klebsiella* spp. ($n = 13$), *Pseudomonas* spp. ($n = 10$), *Candida* spp. ($n = 4$), *Escherichia coli* ($n = 4$), *Acinetobacter baumannii* ($n = 4$), and *Streptococcus pneumoniae* ($n = 4$). Other less clinically relevant isolates included staphylococcal species ($n = 91$) and viridans group streptococci ($n = 16$), which were considered skin contaminants and excluded from analyses. These infections were treated empirically with a wide range of antimicrobial drugs, including six antimicrobials classified as "Reserve" by the Access, Watch and Reserve (AWaRE) classification of antibiotics. AST results showed high resistance rates, including 40% methicillin resistance in *S. aureus*, multidrug-resistant *Klebsiella* spp. (with 20% being carbapenem-resistant), and resistance to third-generation cephalosporins in all *E. coli* isolates. Instances of changes in empiric antimicrobial regimens following microbiology results were very limited.

The project set the foundation for a national AMR surveillance system by implementing microbiological diagnostics, standardizing clinical pathways and increasing clinician awareness of routine microbiology. Reliable AMR data can inform revised treatment guidelines and empiric therapy recommendations, supporting antibiotic stewardship strategies like targeted prescribing and de-escalation to narrower-spectrum antimicrobials. The project also supported the National AMR Centre in its efforts to obtain accreditation in accordance with the international standard International Organization for Standardization standard (ISO) 15189. Extending these practices to additional facilities is recommended, alongside securing resources and introducing internal quality systems in laboratories.

Further recommended actions include expanding microbiological testing capacity, reducing AST turnaround times, and adopting standardized protocols nationwide. Sustainability of this workflow will require continued communication between clinical and laboratory teams and adequate resource allocation.

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INTRODUCTION

Antimicrobial resistance (AMR) is one of the public health threats with the highest burden of disease globally *(1)*. National AMR surveillance is crucial for tackling AMR by providing the basis for developing clinical guidelines, effective infection control measures and the optimization of antimicrobial use. Additionally, it enhances awareness and knowledge of AMR among health-care professionals, policy-makers and the general public *(2)*. Routine microbiological diagnostics, including the detection of bacterial pathogens and antimicrobial susceptibility testing (AST), is a necessary step in the implementation of AMR surveillance. Its underutilization leads to fewer samples being processed, antimicrobial therapy often being prescribed without microbiological evidence and surveillance data being biased towards severe cases or patients experiencing failure of first-line therapy.

Bloodstream infections (BSIs) are caused by bacteria or other organisms entering the bloodstream. BSIs rank among the most serious infections, leading to high mortality rates *(3)*. The underutilization of microbiological diagnostics means that clinicians managing BSIs often continue empiric therapy – typically with broad-spectrum antibiotics – without the ability to shift to targeted treatment based on pathogen identification or de-escalate to narrow-spectrum agents. This approach compromises patient care quality and may induce the emergence of AMR, which exacerbates the already critical problem of BSIs. BSIs caused by resistant organisms have been associated with higher morbidity, higher mortality and a longer hospital stay *(4)*.

In Uzbekistan, AMR poses a critical challenge to public health, having directly caused approximately 4500 deaths and contributed to an additional 17 200 deaths in 2019 *(5)*. The death toll from AMR surpasses that of digestive diseases; respiratory infections including tuberculosis; diabetes; kidney diseases; maternal and neonatal disorders; and unintentional injuries *(5)*. This high mortality rate emphasizes the need for urgent and effective responses to tackle this growing health threat, including improved diagnostics and antibiotic stewardship, public health policies, and health-care practices tailored to the specific needs and challenges faced by Uzbekistan.

The country is a member of the Central Asian and European Surveillance of Antimicrobial Resistance (CAESAR) network. The main priority areas of the plan are building national surveillance for AMR and reporting to CAESAR and the Global Antimicrobial Resistance and Use Surveillance, strengthening research and capacity of the workforce, improving infection prevention and control in health facilities, and raising awareness of AMR in the population. However, full implementation is still in progress, with challenges such as limited surveillance infrastructure, laboratory capacity and availability of specialized staff. Over-the-counter antibiotic sales are still common, worsening the AMR problem. Additionally, insufficient health funding and governance also affect the country's ability to effectively combat AMR.

The Proof-of-principle routine diagnostic project for antimicrobial resistance surveillance in Uzbekistan (PoP project in Uzbekistan), based on the *Proof-of-principle antimicrobial resistance routine diagnostics surveillance project (PoP project): protocol: version 2.0 (6)* was undertaken between 2019 and 2022, focusing on enhancing patient safety by identifying gaps in microbiological diagnostics and promoting the utilization of laboratory testing to inform routine prescribing practices.

The PoP project in Uzbekistan aimed to:

- improve sampling practices, especially where rates were low or samples were only collected after treatment had failed;

- refine national treatment guidelines to encourage BC sampling for patients showing signs of BSI, thus offering insights into national AMR trends;
- promote bacteriological testing, including species identification and AST, to underscore the importance of clinical microbiology in diagnosing suspected BSIs;
- provide the foundation for local, data-driven prescribing guidelines by utilizing local susceptibility data; and
- bolster AMR reference functions and surveillance capabilities at the AMR Centre.

The PoP project in Uzbekistan was conducted across four health-care facilities (HCFs) in the city of Tashkent (Table 1), with their laboratory situated within Uzbekistan's AMR Centre serving the pivotal role of the AMR Centre.

Table 1. Participating HCFs

HCF	Code
Republican Specialized Scientific and Practical Medical Centre for Epidemiology, Microbiology, Infectious Diseases and Parasitic Diseases	HCF 1
City Clinical Infectious Diseases Hospital	HCF 2
City Clinical Hospital	HCF 3
Republican Specialized Scientific Practice Medical Centre of Paediatrics	HCF 4

METHODS

Patients who met the criteria for systemic inflammatory response syndrome (SIRS) *(7)* at the four participating HCFs were eligible for enrolment. Blood samples were collected from patients participating in the project over the three-year project period in line with the PoP project guidelines for blood sampling *(6).* Blood samples were tested using standard microbiological methods for isolation and identification, and AST of clinically significant pathogens was performed. Results were reported first as preliminary results (i.e. Gram staining of positive BC), allowing clinicians to adjust antimicrobial therapy promptly, followed by final results, with species identification and AST. Methodology for phenotypic AST and interpretation of results followed the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines *(8).* Clinical and Laboratory Standards Institute (CLSI) guidelines were used for AST of *Neisseria meningitidis (9).* For quality assurance purposes, isolation and identification of microorganisms were performed in parallel at the HCFs and at the National AMR Centre, except for HCF3, which tested their samples at the AMR Centre only. All AST was performed exclusively at the National AMR Centre. Weekly visits to HCFs ensured strict adherence to diagnostic procedures as per the PoP protocol and standard operating procedures. This included verifying the adherence to the recommended number of BC bottles – four bottles for adults and one/two bottles for children (depending on age) – to improve isolation rate and quality of laboratory results. The results were processed using R version 4.4.1 *(10).* Detailed information on methodology can be found in Annex 1

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RESULTS

CHARACTERISTICS OF THE PROJECT GROUP

During the project period, 2063 cases of suspected BSI admitted at the participating HCFs were enrolled as project participants based on their primary diagnosis, specifically meeting two or more of the systemic inflammatory response syndrome criteria *(7)*.

A majority of project participants (62%) were 5 years old or younger, where newborns (< 28 days old) presented the highest positivity of BC results (23%). Positivity of BC results was lower in older project participants, with the lowest values seen in those 65 years or older (5%). Positive BC results were obtained from 210 out of 1190 males, and 89 out of 873 females (70% and 30% of all positive participants, respectively). The number and percentage of project participants with a positive BC by age group is shown in Table 2.

Table 2. Breakdown of project participants by BC results by age

Age group	Number of participants	Percentage of participants, %	Participants with a positive BC	Percentage of total positive BC, %
< 28 days	89	4.3%	20	6.6%
1–11 months	722	35.0%	105	35.1%
1– 5 years	471	22.8%	72	24.1%
6–17 years	211	10.2%	34	11.4%
18–35 years	254	12.3%	40	13.4%
36–50 years	115	5.6%	13	4.3%
51–65 years	118	5.7%	11	3.7%
> 65 years	83	4.0%	4	1.3%
Total	2 063	100.0%	299	100.0%

The prevailing diagnosis of enrolled project participants with suspected BSIs were sepsis (52.3%) and fetal infection (28.0%) in newborns; sepsis (44.0%) and pneumonia (22%) in the age group from 1 month to 17 years; and pneumonia (26%) and invasive meningococcal infection (16%) in those over 18 years old. Most positive BC were linked to the primary diagnoses identified in the study, with invasive meningococcal infections being a major contributor to positive results in the age group from 1 month to 17 years.

Table 3 shows the number of participants enrolled in each HCF, alongside the number of participants with positive BCs and the number of microbiological tests performed per patient.

Table 3. Breakdown of microbiological tests performed by participating HCFs, February 2019–March 2022.

HCF	Number of patients with suspected BSI (% of total)	Number of patients with positive BCs	Number of microbial isolates[a]
HCF 1	85 (4.1%)	23	24
HCF 2	525 (25.4%)	105	105
HCF 3	339 (16.4%)	16	16
HCF 4	1 114 (54.0%)	155	160
Total	2 063 (100%)	299	305

[a] BCs from six participants (one in HCF 1 and five in HCF 4) yielded two bacterial isolates.

A total of 889 (43%) project participants had received antibiotics in the three months prior to enrolment in the project, of whom 58% had received one antibiotic, 36% two antibiotics and the remainder three antibiotics. Third-generation cephalosporins were the most frequently reported therapies in the three months prior to enrolment in the project (38%), followed by aminoglycosides and fourth-generation cephalosporins (15% and 14%, respectively).

At the time of blood sampling, 1295 (63%) participants were receiving antibiotic treatments, of whom 71% were receiving one antibiotic, 28% were receiving two antibiotics and the remainder were receiving three antibiotics. Third- and fourth-generation cephalosporins accounted for most of the antimicrobial classes among antimicrobial therapies ongoing at the time of sampling (40% and 15%, respectively), followed by penicillins (13%) and carbapenems (8%).

Almost 90% of samples from participants on antibiotics at the time of sampling had negative culture results, compared to 80% of those not on antibiotics. Logistic analysis showed that the number of antimicrobials in ongoing treatment at the time of sampling was significantly associated with negative cultures (odds ratio = 0.66; 95% confidence interval 0.55–0.79). The effect of individual antibiotic classes on sample positivity was not statistically significant, except for phenicols, which were more frequently associated with positive culture results. No significant associations were found between antibiotic use in the three months prior to enrolment and sample positivity.

MICROBIAL SPECIES ISOLATED FROM BCS

A total of 305 microbial isolates were identified in blood samples collected from 299 out of 2063 participants with suspected BSI. Samples from six participants led to the identification of two different microbial species. Species considered clinically relevant accounted for 198 isolates, while 107 isolates belonged to species considered contaminants. The most frequently isolated species were *N. meningitidis* (33%), *S. aureus* (11%), *Enterococcus* spp. (8%), and *K. pneumoniae* (4%) (Table 4). An outbreak of *N. meningitidis* contributed to the large number of isolates belonging to this species. All outbreak cases were treated at HCF2. In addition to well-known BSI pathogens, other findings of questionable clinical relevance included coagulase-negative staphylococci and *S. intermedius* (30%), and *viridans* group streptococci (5%).

Table 4. Microbial pathogens detected in blood samples from participants with suspected BSI

Category	Total (*N* = 305)	*Acinetobacter* spp. (*N* = 4)	*Candida* spp. (*N* = 4)	*E. coli* (*N* = 4)	*Enterococcus* spp. (*N* = 25)	*Klebsiella* spp. (*N* = 13)	*N. meningitidis* (*N* = 101)	*Pseudomonas* spp. (*N* = 10)	*S. aureus* (*N* = 33)	*S. pneumoniae* (*N* = 4)
HCF										
HCF 1	24 (7.9%)	0 (0%)	0 (0%)	0 (0%)	3 (12%)	1 (7.7%)	0 (0%)	1 (10%)	4 (12%)	0 (0%)
HCF 2	105 (34%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	101 (100%)	0 (0%)	2 (6.1%)	2 (50%)
HCF 3	16 (5.2%)	0 (0%)	0 (0%)	3 (75%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	4 (12%)	0 (0%)
HCF 4	160 (52%)	4 (100%)	4 (100%)	1 (25%)	22 (88%)	12 (92%)	0 (0%)	9 (90%)	23 (70%)	2 (50%)
ICU	191 (63%)	3 (75%)	2 (50%)	3 (75%)	10 (40%)	8 (62%)	97 (96%)	6 (60%)	14 (42%)	3 (75%)
Sex										
Female	91 (30%)	2 (50%)	1 (25%)	2 (50%)	11 (44%)	5 (38%)	22 (22%)	1 (10%)	10 (30%)	0 (0%)
Male	214 (70%)	2 (50%)	3 (75%)	2 (50%)	14 (56%)	8 (62%)	79 (78%)	9 (90%)	23 (70%)	4 (100%)
Age group										
Newborn (0–28 days)	21 (6.9%)	1 (25%)	2 (50%)	0 (0%)	2 (8%)	3 (23%)	0 (0%)	2 (20%)	1 (3.0%)	0 (0%)
1–11 months	108 (35%)	1 (25%)	2 (50%)	1 (25%)	12 (48%)	7 (54%)	2 (2.0%)	5 (50%)	15 (45%)	1 (25%)
1–5 years	73 (24%)	2 (50%)	0 (0%)	0 (0%)	9 (36%)	3 (23%)	26 (26%)	2 (20%)	12 (36%)	1 (25%)
6–17 years	34 (11%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	27 (27%)	0 (0%)	1 (3.0%)	1 (25%)
18–35 years	40 (13%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	36 (36%)	0 (0%)	0 (0%)	1 (25%)
36–50 years	13 (4.3%)	0 (0%)	0 (0%)	1 (25%)	0 (0%)	0 (0%)	7 (6.9%)	0 (0%)	2 (6.1%)	0 (0%)
51–65 years	11 (3.6%)	0 (0%)	0 (0%)	2 (50%)	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	1 (3.0%)	0 (0%)
> 65 years	5 (1.6%)	0 (0%)	0 (0%)	0 (0%)	1 (4%)	0 (0%)	2 (2.0%)	1 (10%)	1 (3.0%)	0 (0%)

A majority of bacterial isolates were available for AST (*n* = 280). AST was not performed on *Candida* spp. isolates (*n* = 4), and the remaining 21 isolates could not be tested due to failure to grow upon arrival at the AMR Centre. Twenty-one of the total 25 *Enterococcus* spp. isolates were identified as *Enterococcus faecalis* (*n* = 7) or *Enterococcus faecium* (*n* = 14) prior to AST. Overall, AMR was detected in a significant fraction of the tested bacterial isolates (50%) obtained from suspected BSIs. Over half of the *K. pneumoniae, E. coli, S. pneumoniae,* and *P. aeruginosa* isolates displayed some type of resistance, including 40% of methicillin-resistant *Staphylococcus aureus* among *S. aureus* infections, multidrug-resistant *K. pneumoniae* with 8–50% resistance to carbapenems, and third-generation cephalosporin resistance observed in all *E. coli* isolates. Table 5 shows the number of resistant isolates and total number of isolates tested for each bacterial pathogen and antimicrobial agent combination.

Table 5. Number of resistant isolates out of the total isolates tested for each species-antimicrobial agent combination in bacterial isolates from suspected BSI cases

Class	Antimicrobial	Method	*A. baumannii*	*E. faecalis*	*E. faecium*	*E. coli*	*K. pneumoniae*	*N. meningitidis*			*P. aeruginosa*	*S. aureus*	*Streptococcus pneumoniae*	*Streptococcus* spp.
–	–	–	–	–	–	–	–	Resis-tant	Non-susceptible	Inter-mediate	–	–	–	–
Beta-lactams	Ampicillin	DD	–	RO	RO	4/4	13/13	–	–	–	–	–	–	–
	Penicillin	DD	–	–	–	–	–	–	–	–	–	–	–	–
	Penicillin	ET	–	–	–	–	–	2/4	–	1/4	–	–	0/1	–
	Penicillin	MIC	–	–	–	–	–	–	–	–	–	–	0/1	–
	Piperacillin	DD	–	–	–	–	–	–	–	–	RO	–	–	–
	Oxacillin	DD	–	–	–	–	–	–	–	–	–	1/1	1/2	6/6
Beta-lactams + beta-lactamase inhibitor	Amoxicillin + clavulanic acid	DD	–	–	–	2/2	12/12	–	–	–	–	–	–	–
	Piperacillin + tazobactam	DD	–	–	–	1/3	11/11	–	–	–	RO	–	–	–
Second-generation cephalosporins	Cefoxitin	DD	–	–	–	–	–	–	–	–	–	13/33	–	–
Third-generation cephalosporins	Ceftazidime	DD	–	–	–	4/4	12/13	–	–	–	6/7	–	–	–
	Ceftriaxone	DD	–	–	–	3/3		–	21/85	–	–	–	–	–
	Ceftriaxone	ET	–	–	–			–	1/4	–	–	–	–	–
	Cefotaxime	DD	–	–	–	4/4	12/13	–	18/79	–	–	–	–	–
	Cefotaxime	MIC	–	–	–	–	–	–	–	–	–		0/1	–
Fourth-generation cephalosporins	Cefepime	DD	–	–	–	3/3	13/13	–	–	–	–	–	–	–
Carbapenems	Ertapenem	DD	–	–	–	0/3	3/12	–	–	–	–	–	–	–
	Ertapenem	ET	–	–	–		1/1	–	–	–	–	–	–	–
	Imipenem	DD	2/4	–	–	0/4	2/13	–	–	–	3/6	–	–	–
	Imipenem	ET	0/1	–	–			–	–	–	–	–	–	–
	Meropenem	DD	2/4	–	–	0/4	1/13	–	4/81	–	1/6	–	–	–
	Meropenem	ET	1/2	–	–	–	0/1	–	–	–	–	–	–	–

Fluoroquinolones	Ciprofloxacin	DD	1/4	–	–	4/4	10/13	25/83	–	26/83	2/7	2/21	–	–
	Levofloxacin	DD	0/2	–	–	3/3	3/12	–	–	–	2/7	2/31	0/2	–
	Moxifloxacin	DD	–	–	–	1/1	10/11	–	–	–	–	–	0/2	–
	Norfloxacin	DD	–	–	–	–	–	–	–	–	–	2/3	0/2	5/6
	Ofloxacin	DD	–	–	–	2/2	–	–	–	–	–	–	–	–
Aminoglycosides	Amikacin	DD	2/4	–	–	1/4	10/13	–	–	–	1/6	–	–	–
	Gentamicin high dose	DD	–	4/6	8/9	–	–	–	–	–	–	–	–	–
	Gentamicin	DD	1/4	–	–	0/3	12/13	–	–	–	–	–	–	–
	Tobramycin	DD	3/4	–	–	1/1	–	–	–	–	1/1	–	–	–
Glycopeptides	Vancomycin	DD	–	0/6	0/13	–	–	–	–	–	–	–	–	–
	Vancomycin	ET	–	0/1	0/2	–	–	–	–	–	–	0/12	–	–
	Vancomycin	MIC	–	0/1	–	–	–	–	–	–	–	0/3	–	–
Macrolides	Azithromycin	DD	–	–	–	–	–	–	40/48	–	–	–	–	–
	Erythromycin	DD	–	–	–	–	–	–	–	–	–	14/33	1/2	7/8
Lincosamides	Clindamycin	DD	–	–	–	–	–	–	–	–	–	13/33	0/1	1/8
Tetracyclines	Minocycline	DD	–	–	–	–	–	0/72	–	–	–	–	–	–
	Tigecycline	ET	–	–	–	0/1	–	–	–	–	–	–	–	–
Oxazolidinones	Linezolid	DD	–	0/7	0/14	–	–	–	–	–	–	0/33	–	0/3
Chloramphenicol	Chloram-phenicol	DD	–	–	–	–	–	0/84	–	16/84	–	–	–	–
Polymyxins	Teicoplanin	DD	–	0/6	RO	–	–	–	–	–	–	–	–	–
	Teicoplanin	ET	–	0/1	0/5	–	–	–	–	–	–	–	–	–
	Teicoplanin	MIC	–	0/1	–	–	–	–		–	–	–	–	–
Lipopeptides	Daptomycin	MIC	–	–	–	–	–	–	–	–	–	0/1	–	–
Rifamycins	Rifampicin	DD	–	–	–	–	–	1/83	–	–	–	1/33	–	–
Sulfonamides	Trimetho-prim + sulfa-methoxazole	DD	–	–	–	–	–	42/70	–	10/70	–	–	–	–

Notes: DD: disc diffusion; ET: Etest (Gradient Strip Method); MIC: minimum inhibitory concentration; RO: results omitted (see Annex 2). All results are based on EUCAST AST methods and breakpoints *(8)*, except those for *N. meningitidis* which followed CLSI guidelines with three possible outcomes *(9)*. The AST method employed (DD, ET, MIC) is given for each antimicrobial.

TURNAROUND TIMES FOR PRELIMINARY AND FINAL RESULTS AT HCFS

Across all hospitals, 86% (1780) of blood samples were received by their respective laboratories within 24 hours of sampling, 11% (236) arrived between 24–48 hours, 2% (33) between 48–72 hours, and the remaining 1% (14) took more than 72 hours to reach the laboratory. Laboratory results were reported to the clinic in two steps. Gram staining results were reported first as preliminary results, followed by final laboratory results, which included a diagnosis based on species identification and AST. Most preliminary results (62%) were reported to clinics within two days, whereas final results were more often reported after 4–7 days (Table 6).

Table 6. Turnaround times for reporting preliminary positive results (Gram stain of a positive BC) and final results (species identification and AST) at each HCF

	Reporting time to the clinic (median days and interquartile range)			
–	HCF 1	HCF 2	HCF 3	HCF 4
Days until preliminary report	2 (2–3.5)	3 (2–4)	2 (2–2)	1 (1–2)
Days until final report	7 (6–8)	7 (6–8)	5 (5-6)	5 (5–7)

PRESCRIPTION PRACTICES

Most participants (1892/2063) received empiric antimicrobial therapy, including antimicrobial drugs classified as "Access" (nine different antimicrobials used in total), "Watch" (30 antimicrobials used), and "Reserve" (two antimicrobials used), according to the WHO AWaRe classification of antibiotics for evaluation and monitoring of use, updated in 2023 *(11)* (Table 7). The large amount of "Watch" antimicrobials is associated with the frequent prescription of third-generation cephalosporins, indicated for treatment of meningococcal disease. "Reserve" antimicrobials used included fosfomycin and linezolid. BCs from most of the patients receiving fosfomycin (20/22) were negative. Only one patient treated empirically with fosfomycin changed therapy to cefepime, and subsequently linezolid, after receiving their preliminary and final results, respectively. The four patients treated empirically with linezolid yielded negative BCs and maintained linezolid therapy.

Table 7. Empiric therapy administered to participants by antimicrobial class

Empiric treatment	*N*	%
Cephalosporins (3rd generation)	482	23.4
Beta-lactams/beta-lactamase inhibitors	357	17.3
Beta-lactams	275	13.3
Cephalosporins (4th generation)	253	12.3
Carbapenems	165	8
Cephalosporins (1st/2nd generation)	140	6.8
Chloramphenicol	105	5.1
Fluoroquinolones	39	1.9
Aminoglycosides	33	1.6
Fosfomycin	22	1.1
Glycopeptides	7	0.3
Macrolides	4	0.2
Oxazolidinones	4	0.2
Cephalosporins/beta-lactamase inhibitors	3	0.1
Lincosamides	1	0
Nitrofurans	1	0
Polypeptides	1	0
No empiric treatment	171	8.3
Total	2 063	100

Preliminary microbiological results (Gram staining) led to changes in empiric therapy in 97 participants.
The report of final results (bacterial identification and AST results) led to changes in 27 participants. Additionally, two of the 171 participants that did not receive empiric therapy, initiated treatment after the final results became available.

DISCUSSION

The WHO *Global action plan on antimicrobial resistance (2)* urged countries to establish national surveillance systems to gain insight into AMR trends, identify areas for action, and address challenges at national and regional levels. These surveillance systems also raise clinician awareness of microorganisms and AMR patterns in their regions and hospitals, promoting the adoption of antimicrobial stewardship measures, such as minimizing the use of broad-spectrum agents.

Similar to this project, a recent assessment of a PoP project implemented in Georgia proposed a list of essential elements for developing a laboratory-based AMR surveillance system. These include strong governmental commitment to ensure sustainable financial investment and the establishment of a functional National AMR Centre. Additionally, standardized protocols for sample collection, data analysis, and reporting are essential to ensure consistency and reliability *(12)*. Strengthened laboratory capacity, supported by robust quality management systems and targeted training aligned with established guidelines and standard operating procedures are also necessary. Finally, maintaining an uninterrupted supply of high-quality materials and equipment is crucial for operational continuity. The findings also stressed the importance of showcasing the clinical and public health value of microbiological diagnostics, fostering active case identification, enhancing collaboration between microbiologists and clinicians, and refining data management and analysis practices. Furthermore, the study highlighted the need to balance universal standards with expert guidance and local contexts to accurately capture the epidemiology of BSIs and optimize diagnostic utilization.

This current project demonstrated the feasibility of a systematic workflow for microbiological diagnostics in the routine management of BSIs by successfully implementing standardized blood sampling, laboratory methods, EUCAST guidelines and quality assurance measures. It also streamlined communication between clinicians and microbiology laboratories and strengthened the collection and reporting of standardized, quality-assured AMR data for national surveillance and global reporting. Despite these advancements, questions remain about the laboratory procurement required for sustained microbiological testing and the capacity of facilities to handle high volumes of samples. In addition, the project results suggest the need to improve the timeliness of AST results from BSIs to enable clinicians to de-escalate therapy to narrower spectrum, targeted agents when appropriate.

Sustained and reliable AMR surveillance data will inform the development of empiric therapy guidelines, promoting a targeted approach to common infections. The project revealed widespread use of "Watch" and to some extent "Reserve" antimicrobials, as per the WHO AWaRE classification *(11)*. While this may reflect local resistance patterns and patient needs, the relatively few changes in empiric therapy suggest that the integration of microbiology results into routine clinical decisions is still ongoing and depends on timely turnaround times. Achieving this transformation in empiric therapy practices depends not only on clinician awareness and commitment but also on the availability of appropriate antimicrobial supply to match targeted therapies. Moreover, generating surveillance data based on diagnostics will support the implementation of national infection prevention and control programmes, contributing to more effective health-care practices.
Finally, assessing the mid- and long-term impact of the implemented workflow on AMR patterns in Uzbekistan will require ensuring the sustainability of these efforts, along with continued engagement with local experts to refine and optimize these systems.

BENEFITS OF THE PROJECT

Building on the efforts to strengthen AMR surveillance and antimicrobial stewardship, the PoP project in Uzbekistan led to several key developments that have enhanced clinical and laboratory practices. One of the major achievements was the establishment of multi-disciplinary expert teams at the HCFs, which improved the understanding of the importance of using microbiology to inform prescribing practices. This collaborative approach raised awareness and improved laboratory capacity for species identification and AST at local laboratories.

The project also provided a valuable opportunity for nurses to develop their skills in BC sample collection while adhering to standard protocols. To support these efforts, a guidance document *Biological Sample Collection for Laboratory Testing (13)* was developed and put into practice on a national scale in 2022. This document has helped standardize procedures and ensure consistency in laboratory testing across various health-care settings.

In addition to improving national laboratory practices, the project laid the foundation for developing Uzbekistan's AMR surveillance system. The National AMR Centre played a crucial role in this process by participating in CAESAR's External Quality Assessments *(14)* from 2016 to 2022, which ensured the use of standard laboratory methodology. To further support this development, training workshops on the EUCAST methodology are being conducted annually, providing continuous professional development for laboratory personnel. Additionally, the National AMR Centre received accreditation in line with the international global standard ISO 15189 requirements from the National Accreditation System in 2020 and confirmed its status in 2023 *(15)*.

Importantly, the PoP project in Uzbekistan also facilitated a simulated model of an ideal clinical pathway, demonstrating how clinical practices and laboratory functions can be integrated for optimal patient care. Through collaboration between these integral components, the project showed how a well-coordinated approach can enhance the diagnosis and management of infections, ultimately contributing to better health outcomes.

CHALLENGES OF THE PROJECT

Challenges affecting implementation

The coronavirus disease (COVID-19) pandemic and seasonal outbreaks disrupted the workflow, suggesting the current capacity is not enough to sustain routine work during outbreaks. Frequent staff turnover at the HCFs complicated the data collection process, resulting in the need for repeated training to maintain adherence to protocols. Laboratory challenges also emerged, particularly regarding the procurement of quality reagents for microbiological testing in state laboratories. Without distributors importing high-quality reagents into the country, the project relied on WHO procurement, which encountered delays and customs problems. Another challenge was related to prescribing practices, particularly the extensive prescription of antibiotics prior to hospital admission. This practice was common, complicating diagnosis, and possibly generating a bias on AMR data.

Adherence to protocols

During weekly visits to HCFs, members of the national team coordinating the PoP project in Uzbekistan implementation and activities worked closely with clinic personnel to promote strict adherence to diagnostic procedures according to the PoP protocol and the standard operating procedures for BC sampling. Despite these efforts, difficulties with BC sampling persisted in some groups, especially concerning sample volume and the number of bottles used. While methods generally aligned with the protocol, which is based on the patient's age, adherence to the recommended number of BC bottles varied. Refusal by the parents of paediatric participants, in the case of severe conditions, often led to collecting smaller sample volumes or using fewer bottles than recommended.

Limitations

The project generated important insights into the AMR landscape in Uzbekistan. Among 2063 participants with suspected BSI, 299 positive cultures offered an overview of the most common BSI etiological agents *(16).* However, the large proportion of patients undergoing antimicrobial therapy at the time of sampling (63%) had a significant impact on the number of positive cultures and may have introduced a bias towards the selection of drug-resistant bacteria in the results. Secondly, the distribution of pathogens identified in the project does not reflect the typical aetiology of BSIs. This discrepancy was largely due to an outbreak of *N. meningitidis* during the project, which resulted in 33% of positive samples testing positive to this pathogen, while *N. meningitidis* is typically not a common cause of BSIs outside of outbreak situations *(16,17).* Based on Diekema et al. *(16)* the most common BSI pathogens globally include *S. aureus* (20.7%), *E. coli* (20.5%), *K. pneumoniae* (7.7%), *P. aeruginosa* (5.3%), and *E. faecalis* (5.2%). Frequency of *N. meningitidis* in BSIs is typically less than 0.5% over extended periods, as demonstrated by Hufnagel et al. *(17).* The inclusion of *N. meningitidis* isolates in this project, although not typical for routine AMR surveillance, was justified by the opportunity to evaluate laboratory capacity during a period of increased demand. Including these isolates also helped raise awareness of the benefits of microbiological testing in outbreak scenarios – an aspect that aligns with the broader goals of strengthening diagnostic capacity and responsiveness. Finally, while the project produced valuable AST data, caution should be exercised when extrapolating these results beyond the scope of this project, due to the sample size and limited project period. Additional data are necessary to confirm whether the results are representative of AMR patterns across the country.

Furthermore, some AST results raised quality concerns due to unexpected outcomes or inconsistencies between antimicrobial agents that should have shown aligned results. These findings underscore the need for a robust quality control system. All omitted results are provided in Annex 2.

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RECOMMENDED ACTIONS

RECOMMENDED ACTIONS FOR HCFS

To enhance patient care, hospitals should focus on increasing the collection of samples for microbiological testing and implementing a quality management system. This will help maintain high standards of clinical and laboratory practices and ensure reliable results. In consolidating these practices, the protocols developed under the project should be officially endorsed as regulatory documents, to ensure standardized procedures are consistency followed. Given the large proportion of negative BCs in patients undergoing antibiotic therapy at the time of sampling, HCFs may consider introducing molecular methods in their diagnostic protocols which may increase the sensitivity of detecting specific pathogens and reduce turnaround time.

Regular training should also be provided to nurses and doctors to reinforce the importance of quality sample collection, and to laboratory personnel to ensure adherence to protocols. These training sessions should emphasize the critical role that health-care and laboratory personnel play in obtaining accurate results and how these results directly impact patient care. Furthermore, administrative teams at HCFs should collaborate with clinical staff to ensure the appropriate procurement of quality reagents for microbiological tests, which is essential for maintaining effective laboratory operations.

A well-functioning and high-quality workflow for microbiological testing of BSI cases will have a direct impact on patient care. One of the key benefits of having AST data is the ability to implement pathogen-directed therapy using narrower spectrum antimicrobials. However, for this to be feasible, clinicians require timely AST results, ideally within 1–2 days. In the project, turnaround times were often longer, taking more than 5 days to report. To address this issue, there should be a national effort to optimize laboratory workflows and ensure adequate staffing and training, which will help reduce turnaround times and build clinician confidence in the value of microbiological testing.

Last, given the limited changes in antimicrobial therapy following preliminary and final results, continuous training in antimicrobial stewardship could help clinicians better apply microbiology testing, although other factors may also contribute to the small number of adjustments observed.

AT THE NATIONAL LEVEL

A functional and quality-assured workflow for laboratory diagnosis and AST of BSIs as discussed above sets the foundation of an effective national surveillance network. To establish such a system, it is important to include a wider range of facilities to better represent the health-care system in the country.

When possible, collecting robust AST data should be complemented by other relevant indicators such as mortality rates, intensive care unit admission, hospital stay and antimicrobial consumption. These additional indicators will help identify areas for action and facilitate the introduction and evaluation of interventions.

Finally, the establishment of a sustained surveillance system will provide an accurate picture of AMR in the country. This will enable the development of treatment guidelines based on national evidence and provide the necessary information to obtain and allocate resources effectively where they are needed the most.

REFERENCES[1]

1. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. Lancet. 2022;399(10325):629–655 (https://doi.org/10.1016/S0140-6736(21)02724-0).
2. Global action plan on antimicrobial resistance. Geneva: World Health Organization; 2015 (https://iris.who.int/handle/10665/193736).
3. Goto M, Al-Hasan MN. Overall burden of bloodstream infection and nosocomial bloodstream infection in North America and Europe. Clin Microbiol Infect. 2013;19(6):501–509 (https://doi.org/10.1111/1469-0691.12195).
4. Allel K, Stone J, Undurraga EA, Day L, Moore CE, Lin L, et al. The impact of inpatient bloodstream infections caused by antibiotic-resistant bacteria in low- and middle-income countries: A systematic review and meta-analysis. PLoS Med. 2023;20(6):e1004199 (https://doi.org/10.1371/journal.pmed.1004199).
5. The burden of antimicrobial resistance (AMR) in Uzbekistan. Washington DC: Institute for Health Metrics and Evaluation; 2023 (https://www.healthdata.org/sites/default/files/2023-09/Uzbekistan.pdf).
6. Proof-of-principle antimicrobial resistance routine diagnostics surveillance project (PoP project): protocol: version 2.0. Copenhagen: WHO Regional Office for Europe; 2018 (WHO/EURO:2018-2972-42730-59614; https://iris.who.int/handle/10665/346076).
7. Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest. 1992;101(6):1644–55 (https://doi.org/10.1378/chest.101.6.1644).
8. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0. Basel: European Committee on Antimicrobial Susceptibility Testing; 2024 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf).
9. CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition [website]. CLSI; 2024 (https://clsi.org/standards/products/microbiology/documents/m100/).
10. The R Project for Statistical Computing [website]. The R Foundation; 2024 (https://www.r-project.org/).
11. The selection and use of essential medicines 2023: web annex C: WHO AWaRe "(access, watch, reserve)" classification of antibiotics for evaluation and monitoring of use, 2023. Geneva: World Health Organization; 2023 (https://iris.who.int/handle/10665/371093). License: CC BY-NC-SA 3.0 IGO.
12. Malania L, Wagenaar I, Karatuna O, Tambic Andrasevic A, Tsereteli D, Baidauri M, et al. Setting up laboratory-based antimicrobial resistance surveillance in low- and middle-income countries: lessons learned from Georgia. Clin Microbiol Infect. 2021;27(10):1409–1413 (https://.doi.org/10.1016/j.cmi.2021.05.027).
13. Bektimirov AM, Abdukhalilova G. K. ЛАБОРАТОР ТЕКШИРУВЛАР УЧУН БИОЛОГИК МАТЕРИАЛЛАРНИ ОЛИШ БЎЙИЧА Услубий кўлланма [Methodological guide for obtaining biological materials for laboratory examinations]. Tashkent: Printing house "Glossa"; 2022 (in Uzbek).
14. European Centre for Disease Prevention and Control and WHO Regional Office for Europe. Antimicrobial resistance surveillance in Europe 2023–2021 data. Stockholm: European Centre for Disease Prevention and Control and WHO Regional Office for Europe; 2023 (https://iris.who.int/handle/10665/366822). License: CC BY-NC-SA 3.0 IGO.
15. Uzbekistan. Improving access to quality-assured laboratory services in Uzbekistan [website]. World Health Organization; 2024 (https://www.who.int/about/accountability/results/who-results-report-2020-mtr/country-story/2022/improving-access-to-quality-assured-laboratory-services-in-uzbekistan).
16. Diekema DJ, Hsueh PR, Mendes RE, Pfaller MA, Rolston KV, Sader HS, et al. The Microbiology of Bloodstream Infection: 20-Year Trends from the SENTRY Antimicrobial Surveillance Program. Antimicrob Agents Chemother. 2019; 63(7):e00355-19 (https://doi.org/10.1128/AAC.00355-19).
17. Hufnagel M, Burger A, Bartelt S, Henneke P, Berner R. Secular trends in pediatric bloodstream infections over a 20-year period at a tertiary care hospital in Germany. Eur J Pediatr. 2008;167(10):1149–59 (https://doi.org/10.1007/s00431-007-0651-4).

[1] All references were accessed on 23 May 2025.

ANNEX 1. PROJECT METHODOLOGY

PROJECT PARTICIPANTS AND PROJECT SITES

The project was implemented at the laboratory of the Antimicrobial Resistance Centre in Uzbekistan. Enrolment took place across four sites identified as health-care facilities (HCFs) (see Methods section).

CRITERIA FOR INCLUSION IN THE PROJECT

In line with the Proof-of principal (PoP) project protocol *(1),* the project included patients who met the criteria for suspected sepsis, based on the Systemic Inflammatory Response Syndrome (SIRS) and that of age-appropriate SIRS criteria for paediatric patients. In summary, any patient with a suspected bloodstream infection (BSI) at any time during their hospital stay showing signs including fever, chills, confusion, fast breathing and fast heartbeat, was eligible to become a participant.

SAMPLE SIZE CALCULATION

As recommended in the PoP protocol, the endpoint for estimating the required sample size was the weighted average susceptibility for any one of the antibiotic choices for empiric treatment of a BSI caused by a Gram-negative bacterium. The denominator for calculating the weighted average being the total number of relevant microorganisms isolated. Based on data collected by the Central Asian and European Surveillance of Antimicrobial Resistance in 2015 *(2),* a rough estimate was made regarding the rank-ordering and proportions of non-susceptibility. It was anticipated that approximately two-thirds of all isolates would be Gram-negative.

LABORATORY ANALYSES

At the beginning of the project, clinicians were trained in proper techniques for collecting BC samples from patients suspected of BSI based on SIRS criteria. Following clinical evaluation and confirmation of indication by a physician, blood samples were collected from patients following the standard operating procedure developed for this purpose. Blood samples were collected from the ulnar vein into bottles with the HiCombi bi-phasic system (HI Media Laboratories, India) containing 20 ml of agar and 40 ml of broth, according to the manufacturers' instructions.

BCs were processed at each HCF following conventional microbiological methods for isolation and identification of microbial pathogens. Results were reported in two phases: preliminary results (i.e., Gram staining of positive BC), which were reported to the clinician as soon as these were available to allow antimicrobial therapy adjustments; followed by final results with species identification and antimicrobial susceptibility testing (AST).
Isolation and identification of microorganisms were performed in parallel at the HCFs and the National AMR Centre, except for HCF3, which tested their samples at the AMR Centre only. The AST of clinically significant isolates was conducted exclusively at the AMR Centre. AST was performed using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) disk diffusion method *(3)* with antibiotic discs from (Liofilchem, Italy) and gradient test (E-test, Liofilchem, Italy) to determine minimum inhibitory concentration, with the exception of *Neisseria meningitidis* isolates which were tested following Clinical and Laboratory Standards Institute (CLSI) standards *(4).* AST results were interpreted according to the corresponding EUCAST and CLSI clinical breakpoint tables.

Confirmatory tests were performed in the laboratory of the Antimicrobial Resistance Centre of the Republican Specialized Scientific-Practical Medical Centre of Epidemiology, Microbiology, Infectious Diseases and Parasitic Diseases of Uzbekistan.

DATA ANALYSIS

Data analysis was performed in R version 4.4.1 *(5)*. R packages tidyverse and ggplot2 facilitated summarizing and visualizing results *(6,7)*. Statistical associations between 1) previous/ongoing antimicrobial therapy and the positivity of BC, and 2) previous/ongoing antimicrobial therapy and occurrence of resistance, were assessed by logistic analysis using lme4 *(8)*.

REFERENCES[1]

1. Proof-of-principle antimicrobial resistance routine diagnostics surveillance project (PoP project): protocol: version 2.0. Copenhagen: WHO Regional Office for Europe; 2018 (https://iris.who.int/handle/10665/346076).
2. Central Asian and Eastern European Surveillance of Antimicrobial Resistance: annual report 2016. Copenhagen: WHO Regional Office for Europe; 2016 (https://iris.who.int/handle/10665/344085).
3. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0. Basel: European Committee on Antimicrobial Susceptibility Testing; 2024 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf).
4. CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition [website]. CLSI; 2024 (https://clsi.org/standards/products/microbiology/documents/m100/).
5. R Core Team R: A language and environment for statistical computing. 2013. Foundation for Statistical Computing, Vienna, Austria. (https://www.r-project.org/).
6. Wickham H, Averick M, Bryan J, Chang W, McGowan LDA, François R, et al. Welcome to the Tidyverse. J Open Source Softw. 2019;4(43):1686 (https://joss.theoj.org/papers/10.21105/joss.01686).
7. Wickham H. ggplot2. WIRES Comp Stats. 2011;3(2):180–5 (https://doi.org/10.1002/wics.147).
8. Bates D, Mächler M, Bolker B, Walker S. Fitting Linear Mixed-Effects Models Using lme4. J Stat Softw. 2015;67(1):1–48 (https://doi.org/10.18637/jss.v067.i01).

[1] All references were accessed on 23 May 2025.

ANNEX 2. ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS OMITTED FROM THE RESULTS SECTION

A summary of the antimicrobial susceptibility testing (AST) data omitted from the results section (Table 5 in the main document) is shown in Table A2.1.

Table A2. 1. Omitted AST results

Class	Antimicrobial	Method	*E. faecalis*	*E. faecium*	*P. aeruginosa*
Beta-Lactam	Ampicillin	DD	4/7	11/14	–
Beta-Lactam	Piperacillin	DD	–	–	3/4
Beta-Lactam/Beta-Lactamase Inhibitor	Piperacillin-Tazobactam	DD	–	–	6/7
Polymyxins	Teicoplanin	DD	–	2/13	–

Notes: DD: disk diffusion.

The level of ampicillin (AMP) resistance in *E. faecalis* is unexpectedly high. According to the European Committee on Antimicrobial Susceptibility Testing and the Clinical Laboratory Standards Institute (CLSI) *(1,2), E. faecalis* is typically highly susceptible to ampicillin (expected level of susceptibility, > 99% according to EUCAST, and > 95% according to the CLSI), with resistance expected to be below 5%. In contrast, resistance to AMP is very common in *E. faecium* and is expected to be observed in approximately > 20% of clinical isolates, according to the CLSI. These unexpected results raise concerns about the accuracy of species identification or potential methodological issues in AST.

Additionally, there is a lack of alignment in the susceptibility of *P. aeruginosa* to piperacillin and piperacillin-tazobactam, as all strains resistant to piperacillin-tazobactam should also be resistant to piperacillin, suggesting possible laboratory errors. Last, the presence of two teicoplanin resistant *E. faecium* strains which are both susceptible to vancomycin indicates a discrepancy in AST results since all known resistance mechanisms causing resistance to teicoplanin also effect vancomycin, further suggesting laboratory quality concerns.

REFERENCES[1]

1. Breakpoint tables for interpretation of MICs and zone diameters. Version 14.0. Basel: European Committee on Antimicrobial Susceptibility Testing; 2024 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.pdf).
2. CLSI M100 Performance Standards for Antimicrobial Susceptibility Testing, 34th Edition [website]. CLSI; 2024 (https://clsi.org/standards/products/microbiology/documents/m100/).

[1] All references were accessed on 23 May 2025.

The WHO Regional Office for Europe

The World Health Organization (WHO) is a specialized agency of the United Nations created in 1948 with the primary responsibility for international health matters and public health. The WHO Regional Office for Europe is one of six regional offices throughout the world, each with its own programme geared to the particular health conditions of the countries it serves.

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