Original article

Assessment of the performance of Romanian Public Health Units in identifying and conducting antimicrobial susceptibility testing for healthcare-associated infection-causing pathogens

ALEXANDRA CÎRSTEA[1,2], CONSTANȚA POPA[2], CRISTINA-GABRIELA IVAN[2], GABRIEL-CĂTĂLIN DINULESCU[2], GEORGETA SORESCU[2], IOANA-DIANA DUMITRAN[2], NICOLETA ALINA MAREȘ[3], TATIANA VASSU-DIMOV[1]

[1]Faculty of Biology, University of Bucharest, Romania
[2]Association for Quality in Medical Laboratories (CALILAB), Romania
[3]Carol Davila University of Medicine and Pharmacy, Romania

Abstract   Detecting healthcare-associated infections (HAIs) is crucial as HAIs can pose a significant threat to patient safety, particularly among vulnerable populations such as the elderly, immunocompromised individuals, and those undergoing invasive medical procedures. Also, it is important to monitor the effectiveness of antibiotics and identify the resistance patterns of microorganisms to ensure appropriate treatment options. Bacteriology external quality control programs provide a means for laboratories to assess their performance and identify areas that require improvement. By participating in such programs, laboratories can evaluate their procedures, equipment, and personnel to ensure that they are producing reliable and accurate results because the External Quality Control (EQC) programs are carried out by medical laboratories using identical procedures as those used for handling patient samples. Our investigation centered on the performance of Romanian Public Health Units in identifying bacteria and conducting antimicrobial susceptibility testing in Bacteriology EQC programs associated with some of the most prevalent HAIs, such as blood infections, respiratory infections, and postoperative infections.

✉ *Corresponding author:* Alexandra Cîrstea; email: alexandrairinacirstea@gmail.com

Introduction

The occurrence of HCAIs is a matter of public health concern due to their potential to escalate treatment expenses, reduce quality of life, and raise the risk of morbidity and mortality (E. N. TCHOUAKET & al., 2022 [4]). Antibiotic consumption is the primary factor responsible for the emergence of new mechanisms of microbial resistance, such as multidrug resistance which is a contributing factor to the onset of HAIs. The development of bacterial resistance limits the treatment options for infections, particularly when it comes to healthcare-associated infections. This makes treatment more challenging and increases the risk of unfavorable outcomes (G. A. POPESCU & al., 2020 [1]).

In Romania, the data collected during the years 2018-2019 showed an increase in the consumption of antibiotics compared to 2016 and 2017. Subsequently, the situation in 2020 was significantly influenced by the evolution of the COVID-19 pandemic (G. A. POPESCU & al., 2020 [1]).

Based on statistical reports from most hospitals in Romania, HAIs continue to be a significantly underappreciated condition, with an incidence rate of only 1.04% among all discharged patients in 2020. The incidence of reported HAIs has been continuously rising between 2012 and 2020, with an acceleration observed after 2016. In 2020, the reported cases reached 23.208, which is 2.8 times higher than the minimum recorded in 2012 (G. A. POPESCU & al., 2020 [1]).

Respiratory infections were the most frequently reported in 2020, accounting for 37% of all reported cases, which is twice the number reported in 2019 (G. A. POPESCU & al., 2020 [1]).

Bacterial resistance to antibiotics has increased for most pathogens circulating in hospitals, while remaining relatively stable for germs that are prevalent in the community. This trend can be attributed to the reduction in oral antibiotic consumption, which decreased by 12-13% during 2016-2017 compared to 2015. The resistance level of *Escherichia coli* decreased considerably, and the percentage of *Methicillin-resistant Staphylococcus aureus* (MRSA) stabilized. Although there is an increasing trend in the reporting of infections associated with antimicrobial-resistant microorganisms at the national level, progress remains limited (G. A. POPESCU & al., 2020 [1]). MRSA remains among the top five causes of nosocomial infections as the global prevalence of antibiotic-resistant pathogenic forms of *S. aureus*, including MRSA, is a significant issue in clinical medicine. *S. aureus* is frequently responsible for wound infections following surgery. It also has the potential to cause a large spectrum of illnesses, ranging from minor skin infections to severe conditions like pneumonia, meningitis, and sepsis (F. TUTULESCU & al., 2018 [9]).

Also, the utilization of carbapenems for treating infections caused by Gram-negative bacteria that are resistant to multiple antibiotics, particularly those producing extended-spectrum beta-lactamase (ESBL) or non-fermentative bacilli such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* (except for ertapenem), has resulted in the emergence of *Enterobacteriaceae* that are resistant to them. The main concern related to the emergence of these bacterial strains is the increase in the difficulty to treat severe infections caused by them (including those that carry a risk of mortality) as only a limited number of antibiotics are effective against them (G.A. POPESCU & al., 2016 [2]). *Pseudomonas aeruginosa* is a dominant pathogen in people suffering from cystic fibrosis, contributing significantly to morbidity and mortality. Its remarkable adaptability greatly enhances its potential to cause chronic infections in such individuals (I. JURADO-MARTÍN & al., 2021 [3]).

The exponential rise in infections caused by *Enterobacterales, particularly Klebsiella pneumoniae* and *Escherichia coli*, is one of the primary concerns associated with antimicrobial resistance (E. MACHADO & al., 2022 [6]). *Klebsiella pneumoniae* is a type of Gram-negative bacterium that is significant in human pathology as it is responsible for causing a broad spectrum of infections such as urinary and respiratory infections, as well as systemic, digestive, and meningeal infection (L. GIUBELAN & al., 2021 [5]).

Serratia marcescens can lead to various types of infections in both adults and children (such as pneumonia, sepsis, wound infection, meningitis, endocarditis, ocular infections, etc.), including opportunistic and nosocomial infections. The entry point for infections with *S. marcescens* are the urinary catheter, intubation, and the central venous catheter. Catheter colonization increases bacterial survival, particularly in biofilm communities. Therefore, biofilms increase resistance to antibiotics (R. ZIVKOVIC ZARIC & al., 2022 [7]).

Vancomycin-resistant Enterococcus spp. (VRE) has emerged as a significant multidrug-resistant nosocomial pathogen and a primary cause of HAIs, over the past 25 years. Patients who are severely ill and immunocompromised are particularly vulnerable to the threat posed by VRE. Its natural characteristics, including innate and acquired resistance to various antimicrobial drug classes, result in limited therapeutic options and this contributes to the threat it poses (A. JANJUSEVIC & al., 2022 [8]).

The purpose of this study is to form a viewpoint on the proficiency of Romanian Public Health Units in identifying bacteria and conducting antimicrobial susceptibility testing

for certain bacteria that cause healthcare - associated infections: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Serratia marcescens.*

Materials and methods

Our research was focused on examining seven of the primary bacteria that are responsible for the prevalence of HAIs worldwide. We utilized the outcomes from an EQC provider. The study was centered on the results registered from Public Health Units in Romania participating to five EQC bacteriology schemes: i) screening for the detection of multidrug-resistant bacteria, ii) bacteriological examination of blood culture, iii) bacteriological examination of sputum, iv) bacteriological examination of nasal secretion and v) bacteriological examination of purulent secretion. For the screening for the detection of multidrug-resistant bacteria EQC program we used the outcomes registered in 2020 –

2022, whereas for the bacteriological examination of blood culture, sputum, nasal secretion, and purulent secretion we utilized the outcomes registered in 2021 and 2022.

Besides the screening for the detection of multidrug-resistant bacteria program, each EQC scheme includes two components for evaluation: identification and susceptibility testing. The screening for the detection of multidrug-resistant bacteria EQC program is designed to only receive the name of the multidrug – resistant bacteria (MDR bacteria) from the participating laboratories.

Results

During the initial phase of the study, our focus was on bacterial species identification from bacteriological examination of blood culture, bacteriological examination of sputum, bacteriological examination of nasal secretion and bacteriological examination of purulent secretion. We centralized the number of results and the percentages of satis-

Table 1 - Bacteriological examination of blood culture

EQC Round	Strain	Number of results obtained by Public Health Units	Percentage of satisfactory results registered (%)
March 2021	*Methicillin-resistant Staphylococcus aureus*	12	100.00%
May 2021	*Pseudomonas aeruginosa*	21	100.00%
September 2021	*Escherichia coli*	25	100.00%
October 2021	*Serratia marcescens*	16	100.00%
November 2021	*Staphylococcus aureus*	26	100.00%
March 2022	*Serratia marcescens*	16	100.00%
April 2022	*Escherichia coli*	5	100.00%
May 2022	*Staphylococcus aureus*	22	90.91%
September 2022	*Klebsiella pneumoniae*	31	100.00%
November 2022	*Escherichia coli*	29	100.00%

Table 2 - Bacteriological examination of sputum

EQC Round	Strain	Number of results obtained by Public Health Units	Percentage of satisfactory results registered (%)
March 2021	*Klebsiella pneumoniae*	15	100.00%
April 2021	*Staphylococcus aureus*	5	100.00%
October 2021	*Pseudomonas aeruginosa*	16	100.00%
November 2021	*Klebsiella pneumoniae*	42	100.00%
March 2022	*Pseudomonas aeruginosa*	21	100.00%
May 2022	*Staphylococcus aureus*	42	100.00%
September 2022	*Methicillin-resistant Staphylococcus aureus*	60	100.00%
October 2022	*Klebsiella pneumoniae*	25	100.00%
November 2022	*Klebsiella pneumoniae*	52	100.00%

Table 3 - Bacteriological examination of nasal secretion

EQC Round	Strain	Number of results obtained by Public Health Units	Percentage of satisfactory results registered (%)
April 2021	*Staphylococcus aureus*	17	100.00%
September 2021	*Staphylococcus aureus*	153	100.00%
October 2021	*Methicillin-resistant Staphylococcus aureus*	78	100.00%
May 2022	*Staphylococcus aureus*	155	100.00%
October 2022	*Staphylococcus aureus*	58	100.00%
November 2022	*Staphylococcus aureus*	176	100.00%

Table 4 - Bacteriological examination of purulent secretion

EQC Round	Strain	Number of results obtained by Public Health Units	Percentage of satisfactory results registered (%)
September 2021	*Escherichia coli*	150	100.00%
October 2021	*Acinetobacter baumannii*	62	93.55%
March 2022	*Pseudomonas aeruginosa*	89	100.00%
April 2022	*Acinetobacter baumannii*	67	98.51%
November 2022	*Escherichia coli*	158	100.00%

An overview of the results reported for bacteria identification between 2021 and 2022
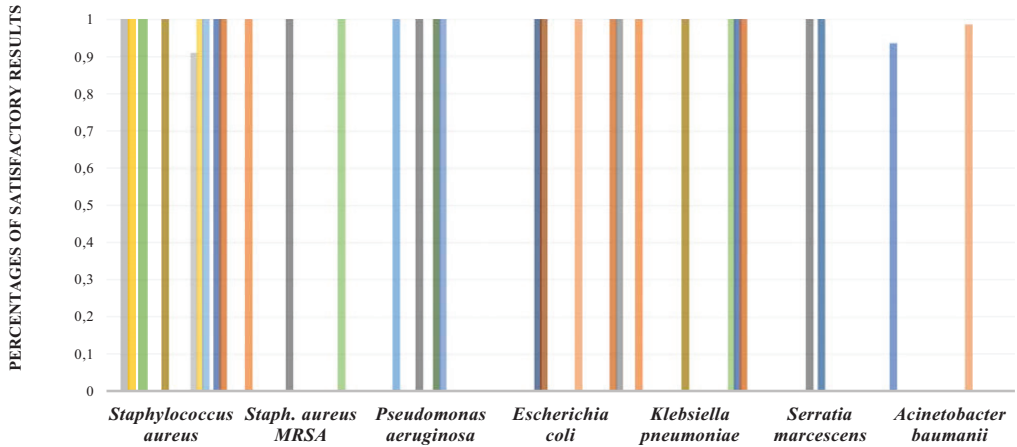
Fig. 1 – An overview of the results obtained by Public Health Units participating on EQC programs – bacterial identification

factory results obtained by the Public Health Units laboratories.

Centralizing the results from the bacteriological examination of blood culture program we observed that nine out of ten EQC rounds analyzed recorded 100% satisfactory results. Only one round registered 90.91% for *S. aureus* (Table 1).

Upon analyzing the outcomes recorded for the bacteriological examination of sputum scheme, we found that in each EQC round, the percentage of satisfactory results was 100% (Table 2).

Upon analyzing the outcomes obtained from the six EQC rounds of bacteriological examination of nasal secretion, we found a similar situation where the percentage of satisfactory results was 100% (Table 3).

Regarding the bacteriological examination of purulent secretion program, our study uncovered that three EQC rounds recorded 100% satisfactory results, while the remaining two registered 93.55% and 98.51%, both for *A. baumannii* (Table 4).

By centralizing the percentages of satisfactory results, we were able to determine that out of the thirty EQC rounds conducted for the four bacteriology programs, the lowest percentage of satisfactory results was 90.91%. This result was recorded for a *S. aureus* strain provided in May 2022 in the Bacteriological examination of blood culture program.

Furthermore, out of the total EQC rounds conducted, only three recorded a percentage of satisfactory results lower than 100%. One of these was for the *S. aureus* strain provided in May 2022, while the other two were recorded in the

Bacteriological examination of purulent secretion program for *Acinetobacter baumannii* in October 2021 (93.55%) and April 2022 (98.51%) (Figure 1).

For the next stage of our study, we directed our attention to the techniques utilized by the Public Health Units laboratories to identify bacteria in the thirty EQC rounds analyzed in the first phase of our research.

A total of 1644 results were reported in 2021 – 2022 for the bacteriological examination of blood culture, sputum, nasal secretion and purulent secretion scheme. The number of results obtained utilizing manual methods was significantly higher (1086) than the number of results obtained using automated systems (558) (Table 5).

The subsequent phase of the study was focused on antimicrobial susceptibility testing. Our initial step was to centralize the outcomes and percentages of satisfactory results recorded for the same four EQC bacteriology programs, i.e. bacteriological examination of blood culture, sputum, nasal secretion, and purulent secretion. During this phase, we conducted the analysis on each bacteria strain provided by the EQC organizer to the four EQC programs.

After centralizing the percentages of satisfactory results, we found that out of the forty *S. aureus* EQC rounds performed for the four bacteriology programs, the lowest percentage of satisfactory results was 90.00%, which was recorded in May 2022 for penicillin in the bacteriological examination of blood culture program. Furthermore, only sixteen cases recorded percentages of satisfactory results lower than 100%, ranging from 90.00% to 99.42%. The remaining 24 cases resulted in a 100% satisfactory outcome.

Table 5 – A viewpoint of the techniques utilized by the Public Health Units laboratories for bacterial species identification

EQC Program	Strain	Number of results obtained using manual method	Number of results obtained using automated systems
Blood culture March 2021	*Methicillin-resistant Staphylococcus aureus*	4	8
Blood culture May 2021	*Pseudomonas aeruginosa*	8	13
Blood culture September 2021	*Escherichia coli*	9	16
Blood culture October 2021	*Serratia marcescens*	5	11
Blood culture November 2021	*Staphylococcus aureus*	9	17
Blood culture March 2022	*Serratia marcescens*	5	11
Blood culture April 2022	*Escherichia coli*	1	4
Blood culture May 2022	*Staphylococcus aureus*	6	16
Blood culture September 2022	*Klebsiella pneumoniae*	11	20
Blood culture November 2022	*Escherichia coli*	10	19
Sputum March 2021	*Klebsiella pneumoniae*	8	7
Sputum April 2021	*Staphylococcus aureus*	4	1
Sputum October 2021	*Pseudomonas aeruginosa*	8	8
Sputum November 2021	*Klebsiella pneumoniae*	18	24
Sputum March 2022	*Pseudomonas aeruginosa*	12	9
Sputum May 2022	*Staphylococcus aureus*	24	18
Sputum September 2022	*Methicillin-resistant Staphylococcus aureus*	30	30
Sputum October 2022	*Klebsiella pneumoniae*	13	12
Sputum November 2022	*Klebsiella pneumoniae*	27	25
Nasal secretion April 2021	*Staphylococcus aureus*	11	6
Nasal secretion September 2021	*Staphylococcus aureus*	117	36
Nasal secretion October 2021	*Methicillin-resistant Staphylococcus aureus*	65	13
Nasal secretion May 2022	*Staphylococcus aureus*	123	32
Nasal secretion October 2022	*Staphylococcus aureus*	40	18
Nasal secretion November 2022	*Staphylococcus aureus*	135	41
Purulent secretion September 2021	*Escherichia coli*	110	40
Purulent secretion October 2021	*Acinetobacter baumannii*	44	18
Purulent secretion March 2022	*Pseudomonas aeruginosa*	70	19
Purulent secretion April 2022	*Acinetobacter baumannii*	47	20
Purulent secretion November 2022	*Escherichia coli*	112	46
Total number of results for each technique		Total number of results = 1086	Total number of results = 558

Legend: S = Susceptible

Table 6 -The performance of Public Health Units regarding antimicrobial susceptibility testing of *Staphylococcus aureus* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Gentamicin	Blood culture - November 2021	S	100.00%
	Blood culture - May 2022	S	95.45%
	Sputum - May 2022	S	100.00%
	Nasal secretion - April 2021	S	100.00%
	Nasal secretion - September 2021	S	99.30%
	Nasal secretion - May 2022	S	100.00%
	Nasal secretion - October 2022	S	98.21%
	Nasal secretion - November 2022	S	99.41%
Cefoxitin	Blood culture - November 2021	S	100.00%
	Blood culture - May 2022	S	90.91%
	Sputum - April 2021	S	100.00%
	Sputum - May 2022	S	100.00%
	Nasal secretion - April 2021	S	100.00%
	Nasal secretion - September 2021	S	99.27%
	Nasal secretion - May 2022	S	100.00%
	Nasal secretion - October 2022	S	100.00%
	Nasal secretion - November 2022	S	100.00%
Penicillin	Blood culture - November 2021	S	100.00%
	Blood culture - May 2022	S	90.00%
	Sputum - April 2021	S	100.00%
	Sputum - May 2022	S	92.31%
	Nasal secretion - April 2021	S	100.00%
	Nasal secretion - September 2021	S	95.68%
	Nasal secretion - November 2022	S	96.79%
Antibiotics	**EQC Program**	**Expected result**	**Percentage of satisfactory results (%)**

	Blood culture - November 2021	S	100.00%
	Blood culture - May 2022	S	95.00%
	Sputum - April 2021	S	100.00%
Erythromycin	Sputum - May 2022	S	100.00%
	Nasal secretion - April 2021	S	100.00%
	Nasal secretion - September 2021	S	97.24%
	Nasal secretion - November 2022	S	98.82%
	Blood culture - November 2021	S	100.00%
	Blood culture - May 2022	S	90.91%
	Sputum - April 2021	S	100.00%
Clindamycin	Sputum - May 2022	S	100.00%
	Nasal secretion - April 2021	S	100.00%
	Nasal secretion - September 2021	S	99.31%
	Nasal secretion - November 2022	S	99.42%
	Blood culture - November 2021	S	100.00%
Linezolid	Blood culture - May 2022	S	100.00%
	Sputum - April 2021	S	100.00%
	Nasal secretion - September 2021	S	100.00%
Trimethoprim - Sulfamethoxazole	Sputum - May 2022	S	100.00%
	Nasal secretion - October 2022	S	100.00%

Table 7 - The performance of Public Health Units regarding antimicrobial susceptibility testing of methicillin-resistant *Staphylococcus aureus* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
	Blood culture - March 2021	R	100%
Gentamicin	Sputum - September 2022	R	98.31%
	Nasal secretion - October 2021	R	92.96%
	Blood culture - March 2021	R	100%
Cefoxitin	Sputum - September 2022	R	100%
	Nasal secretion - October 2021	R	91.89%
	Blood culture - March 2021	R	100%
Penicillin	Sputum - September 2022	R	100%
	Blood culture - March 2021	R	100%
Erythromycin	Sputum - September 2022	R	100%
	Nasal secretion - October 2021	R	95.95%
	Blood culture - March 2021	R	100%
Clindamycin	Sputum - September 2022	R	100%
	Nasal secretion - October 2021	R	97.30%
Linezolid	Blood culture - March 2021	S	100%
	Nasal secretion - October 2021	S	100%
Trimethoprim - Sulfamethoxazole	Sputum - September 2022	S	100%
Vancomycin	Sputum - September 2022	S	100%

Legend: S = Susceptible; R = Resistant

Linezolid and trimethoprim – sulfamethoxazole were the only antibiotics with 100% satisfactory results in every EQC round (Table 6).

For the eighteen *methicillin-resistant Staphylococcus aureus* EQC rounds performed the study revealed that the lowest percentage of satisfactory results was 91.89% recorded for cefoxitin in October 2021 in the bacteriological examination of nasal secretion EQC program. Thirteen out of the eighteen EQC rounds conducted recorded 100% satisfactory results. On the other hand, five EQC rounds registered percentages of satisfactory results ranging from 91.89% to 98.31%. Penicillin, linezolid, trimethoprim– sulfamethoxazole and vancomycin were the antibiotics with 100% satisfactory results in every EQC round (Table 7).

After centralizing the percentages of satisfactory results, we observed that for the *P. aeruginosa* EQC rounds performed for three of the bacteriology programs, the lowest percentage of satisfactory results was 60.00%, which was recorded in March 2022 for ceftriaxone in the Bacteriological examination of sputum program. Five of the nine EQC rounds conducted recorded 100% satisfactory results. Four EQC rounds registered percentages of satisfactory results ranging lower than 100%, from 60.00% to 98.75%. Gentamicin, tobramycin, and amikacin registered 100% satisfactory results in each of the EQC rounds conducted (Table 8).

Table 8 - The performance of Public Health Units regarding antimicrobial susceptibility testing of *Pseudomonas aeruginosa* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Gentamicin	Blood culture - May 2021	S	100.00%
	Sputum - March 2022	S	100.00%
Tobramycin	Blood culture - May 2021	S	100.00%
Ceftriaxone	Sputum - October 2021	R	80.00%
	Sputum - March 2022	R	60.00%
	Purulent secretion - March 2022	R	63.41%
Amikacin	Sputum - October 2021	S	100.00%
Meropenem	Sputum - March 2022	S	100.00%
	Purulent secretion - March 2022	S	98.75%

Legend: S = Susceptible; R = Resistant

Table 9 - The performance of Public Health Units regarding antimicrobial susceptibility testing of *Escherichia coli* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Ampicillin	Blood culture - September 2021	R	100.00%
	Blood culture - April 2022	R	100.00%
	Purulent secretion - September 2021	R	95.49%
	Purulent secretion - November 2022	S	97.58%
Ceftazidime	Blood culture - September 2021	S	100.00%
	Purulent secretion - September 2021	S	100.00%
Gentamicin	Blood culture - September 2021	S	100.00%
	Blood culture - April 2022	S	100.00%
	Purulent secretion - September 2021	S	100.00%
	Purulent secretion - November 2022	S	100.00%
Trimethoprim - Sulfamethoxazole	Blood culture - September 2021	S	100.00%
	Purulent secretion - September 2021	S	99.30%
Amoxicillin - clavulanate	Blood culture - September 2021	S	100.00%
	Blood culture - April 2022	S	100.00%
	Purulent secretion - September 2021	S	88.98%
	Purulent secretion - November 2022	S	98.56%
Ertapenem	Blood culture - September 2021	S	100.00%
	Blood culture - April 2022	S	100.00%
	Purulent secretion - September 2021	S	100.00%
Ceftriaxone	Blood culture - September 2021	S	100.00%
	Blood culture - April 2022	S	100.00%
	Purulent secretion - September 2021	S	100.00%
	Purulent secretion - November 2022	S	100.00%
Levofloxacin	Blood culture - April 2022	S	100.00%
Cefuroxime	Blood culture - April 2022	S	100.00%
Ciprofloxacin	Purulent secretion - November 2022	S	100.00%

Legend: S = Susceptible; R = Resistant

Table 10 - The performance of Public Health Units regarding antimicrobial susceptibility testing of *Serratia marcescens* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Piperacillin – Tazobactam	Blood culture - October 2021	S	100.00%
Gentamicin	Blood culture - October 2021	S	100.00%
Levofloxacin	Blood culture - October 2021	S	100.00%
Meropenem	Blood culture - October 2021	S	100.00%
Amoxicillin – Clavulanic acid	Blood culture - October 2021	R	100.00%
Ceftriaxone	Blood culture - March 2022	S	100.00%
Ampicillin	Blood culture - March 2022	R	100.00%
Cefepime	Blood culture - March 2022	S	100.00%
Ciprofloxacin	Blood culture - March 2022	S	100.00%
Amikacin	Blood culture - March 2022	S	100.00%
Ertapenem	Blood culture - March 2022	S	100.00%
Cefuroxime	Blood culture - March 2022	R	100.00%

Legend: S = Susceptible; R = Resistant

For *E. coli* EQC rounds the study showed that the lowest percentage of satisfactory results was 88.98% recorded for amoxicillin - clavulanate in September 2021 for the bacteriological examination of purulent secretion scheme. Twenty-six EQC rounds were conducted for *E. coli* and twenty of them recorded 100% satisfactory results. Ceftazidime, gentamicin, ertapenem, ceftriaxone, levofloxacin, cefuroxime and ciprofloxacin registered 100% satisfactory results in each of the EQC rounds conducted (Table 9).

After centralizing the percentages of satisfactory results for *S. marcescens* EQC rounds conducted in October 2021 and March 2022 in the bacterial examination of blood culture program, we found that each antibiotic recorded 100% satisfactory results (Table 10).

For the twenty-six *K. pneumoniae* EQC rounds performed for Bacteriological examination of sputum the study revealed that the lowest percentage of satisfactory results was 76.92% recorded for amoxicillin - clavulanate in March 2021. Twenty - two EQC rounds recorded 100% satisfactory results. On the other hand, four rounds registered lower percentages of satisfactory results ranging from 76.92% to 96.00%. ESBL, ceftazidime, meropenem, ertapenem, trimethoprim –sulfamethoxazole, ceftriaxone, levofloxacin, and amikacin were the cases with 100% satisfactory results in every EQC round (Table 11)

After centralizing the results obtained from *A. baumannii* susceptibility testing we observed that there is no EQC round with 100% satisfactory results recorded. The lowest

Table 11 - The performance of Public Health Units regarding antimicrobial susceptibility testing of *Klebsiella pneumoniae* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Ampicillin	Sputum - March 2021	R	93.33%
	Sputum - November 2021	R	100.00%
	Sputum - October 2022	R	100.00%
	Sputum - November 2022	R	100.00%
Amoxicillin - clavulanate	Sputum - March 2021	S	76.92%
	Sputum - November 2021	S	100.00%
	Sputum - October 2022	S	96.00%
ESBL	Sputum - March 2021	Positive	100.00%
	Sputum - October 2022	Negative	100.00%
	Sputum - November 2022	Negative	100.00%
Ceftazidime	Sputum - March 2021	R	100.00%
	Sputum - November 2021	S	100.00%
Ciprofloxacin	Sputum - March 2021	S	80.00%
	Sputum - October 2022	S	100.00%
	Sputum - November 2022	S	100.00%
Meropenem	Sputum - March 2021	S	100.00%
Ertapenem	Sputum - November 2021	S	100.00%
	Sputum - October 2022	S	100.00%
	Sputum - November 2022	S	100.00%
Trimethoprim - Sulfamethoxazole	Sputum - November 2021	S	100.00%
	Sputum - November 2022	S	100.00%
Ceftriaxone	Sputum - November 2021	S	100.00%
	Sputum - October 2022	S	100.00%
Levofloxacin	Sputum - November 2021	S	100.00%
Amikacin	Sputum - October 2022	S	100.00%
	Sputum - November 2022	S	100.00%

Legend: S = Susceptible; R = Resistant

Table 12 - The performance of Public Health Units regarding antimicrobial susceptibility testing of *Acinetobacter baumannii* strains

Antibiotics	EQC Program	Expected result	Percentage of satisfactory results (%)
Levofloxacin	Purulent secretion - October 2021	S	91.38%
	Purulent secretion - April 2022	S	94.92%
Ceftriaxone	Purulent secretion - October 2021	R	68.75%
	Purulent secretion - April 2022	R	71.43%
Amikacin	Purulent secretion - October 2021	S	95.35%
Meropenem	Purulent secretion - October 2021	S	94.64%
Amoxicillin - clavulanate	Purulent secretion - October 2021	R	84.62%
Ampicillin	Purulent secretion - April 2022	R	97.44%
Imipenem	Purulent secretion - April 2022	S	92.98%

Legend: S = Susceptible; R = Resistant

Susceptibility testing - bacterial examination for nasal secretion in September 2022
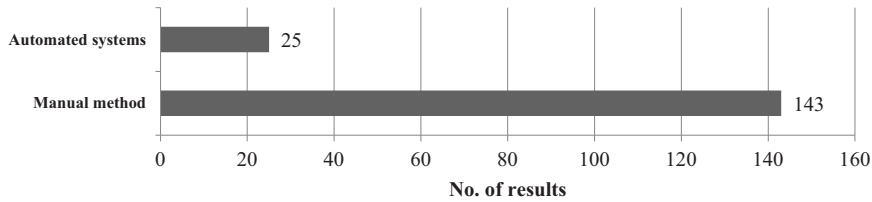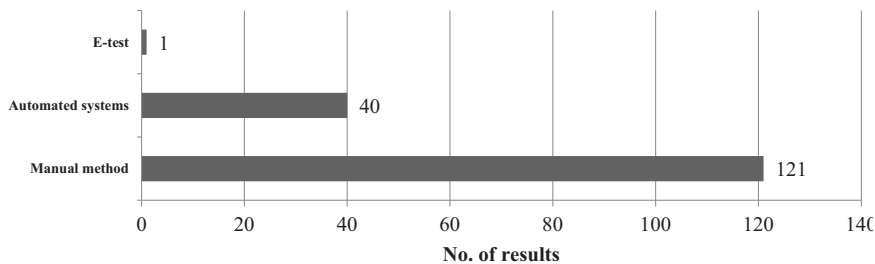

Figure 2 – An overview of the techniques used by Public Health Units laboratories for antimicrobial susceptibility testing on Bacterial examination for nasal secretion EQC program in Septembrer 2022

Susceptibility testing - bacterial examination for purulent secretion in November 2022

Figure 3 – An overview of the techniques used by Public Health Units laboratories for antimicrobial susceptibility testing on Bacterial examination for purulent secretion EQC program in November 2022

percentage was 68.75% registered for ceftriaxone in October 2021. The highest percentage of satisfactory results was 97.44% registered for ampicillin in April 2022 (Table 12).

During this phase of the study, we noted that the only case where percentages of satisfactory results were lower than 100% for all tested antibiotics was in the *A. baumannii* EQC rounds. On the other hand, *Serratia marcescens* EQC program was the only one with 100% percentages of satisfactory results in all cases.

Ceftriaxone, amoxicillin-clavulanate, and ciprofloxacin susceptibility testing recorded the lowest percentages of satisfactory results, which were below 90.00%.

Among the antibiotics tested in the presented EQC rounds, there are some antibiotics to which the strains provided to the laboratories show intrinsic resistance, thus, we have opted to carry out a more detailed analysis on this topic.

We started by identifying the microorganisms that are intrinsically resistant to certain antibiotics and we identified: *P. aeruginosa* intrinsically resistant to ceftriaxone (with 80.00%, 60.00% and 63.41% satisfactory results registered); *S. marcescens* intrinsically resistant to amoxicillin – clavulanate, ampicillin and cefuroxime (all three antibiotics with 100.00% satisfactory results registered); *K. pneumoniae* intrinsically resistant to am-

picillin (with 93.33% and 100.00% satisfactory results registered) and *A. baumannii* intrinsically resistant to amoxicillin – clavulanate (with 84.62% satisfactory results registered) and ampicillin (with 97.44% satisfactory results registered).

One contributing factor to the low percentage of satisfactory results for *A. baumannii* is the incorrect identification of the microorganism (Table 4), leading to inaccurate results for susceptibility testing. On the other hand, for all the other strains provided, in each case 100% satisfactory results were recorded (Tables no. 1, 2 and 4).

According to the procedure of a microbiology laboratory, the intrinsic resistance is reported directly, without the need to test the susceptibility to the respective antibiotic. Since our research also revealed percentages of less than 100% satisfactory results in the case of intrinsic resistance, it turns out that the personnel of some of the laboratories participating in the EQC rounds do not have knowledge about the intrinsic resistance of microorganisms.

We conducted an analysis about the techniques used by Public Health Units laboratories for antimicrobial susceptibility testing (Figure 2 and 3) using the outcomes from Bacterial examination for nasal secretion program in September 2022 and bacterial examination for purulent secretion in November 2022. We only selected the data from September

Table 13 - Bacteriology - screening for the detection of multidrug-resistant bacteria – satisfactory results recorded between 2020 and 2022

EQC Round	Strain	Total number of results registered from Public Health Units	Percentage of satisfactory results registered from Public Health Units
March 2020	MRSA (Methicilin-resistant Staphylococcus aureus)	15	100.00%
June 2020	Klebsiella sp/pneumoniae ESBL positive	18	83.33%
September 2020	VRE (Vancomycin-resistant Enterococcus)	19	94.73%
November 2020	MRSA (Methicilin-resistant Staphylococcus aureus)	17	94.12%
March 2021	VRE (Vancomycin-resistant Enterococcus)	18	83.33%
May 2021	Klebsiella sp/pneumoniae ESBL positive	24	91.67%
September 2021	MRSA (Methicilin-resistant Staphylococcus aureus)	29	100.00%
November 2021	Klebsiella sp/pneumoniae ESBL positive	22	100.00%
March 2022	MRSA (Methicilin-resistant Staphylococcus aureus)	15	100.00%
May 2022	Absence of MDR bacteria	25	88.00%
September 2022	VRE (Vancomycin-resistant Enterococcus)	38	86.84%
November 2022	Absence of MDR bacteria	19	100.00%

and November 2022 since these EQC rounds had the highest number of participating laboratories during the 2021-2022 period.

According to the analysis, the number of laboratories that used automated systems for antimicrobial susceptibility testing was considerably low compared to the number of results obtained using manual methods, in both cases. However, the number of results obtained utilizing automated systems experienced a slight increase in November 2022.

The next stage of the study was conducted analyzing the outcomes recorded for Bacteriology - screening for the detection of multidrug-resistant bacteria EQC program (Table 13).

The research showed that the highest percentages of satisfactory results (100.00%) were recorded in the EQC programs with *MRSA, Klebsiella sp/pneumoniae ESBL positive, "Absence of MDR bacteria".* On the other hand, the lowest

percentages were recorded for *VRE and for ESBL-positive Klebsiella sp./pneumoniae,* 83.33% (Figure 4).

In general, the most favorable outcomes were observed for MRSA testing, while VRE testing produced the least satisfactory results (with no cases of 100.00% satisfactory results recorded).

Similar to earlier stages, we aimed to examine the methods utilized by laboratories for this EQC program from a particular perspective.

TFor the bacteriology - screening for the detection of multidrug-resistant bacteria EQC program in 2020 – 2022 a total of 259 results were reported, out of which 241 were satisfactory, while only 18 were unsatisfactory. 110 satisfactory results were obtained using automated systems and 131 were obtained using manual methods. Regarding the unsatisfactory results, 5 of them were obtained using auto-
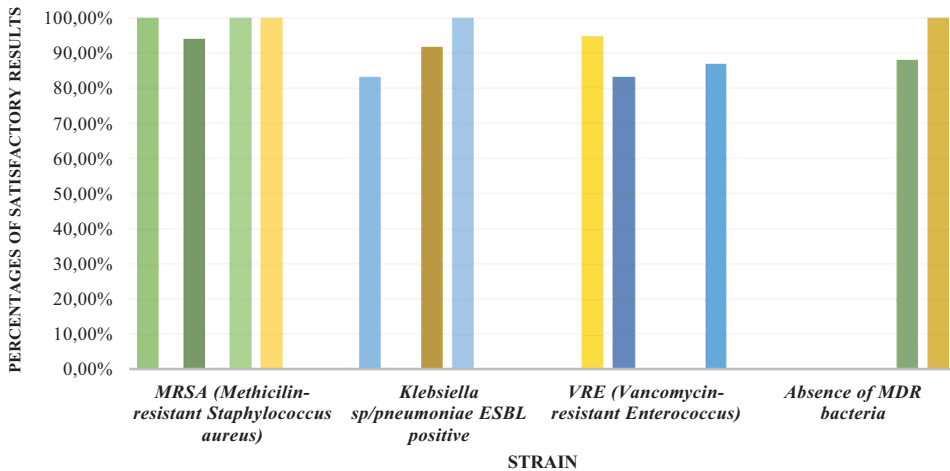

Figure 4 - Satisfactory results recorded between 2020 and 2022 on the bacteriology - screening for the detection of multidrug-resistant bacteria EQC program

Table 14 – An overview of the techniques used by the Romanian Public Health Units participating in Bacteriology - screening for the detection of multidrug-resistant bacteria EQC program in 2020 - 2022

EQC Round	Number of satisfactory results	The method used by Public Health Units with satisfactory results	Number of unsatisfactory results	The method used by Public Health Units with unsatisfactory results
March 2020	15	Automated systems = 7 results Manual method = 8 results	-	-
June 2020	15	Automated systems = 8 results Manual method = 7 results	3	Manual method = 3 results
September 2020	18	Automated systems = 9 results Manual method = 9 results	1	Manual method = 1 result
November 2020	16	Automated systems = 6 results Manual method = 10 results	1	Automated systems = 1 result
March 2021	15	Automated systems = 6 results Manual method = 9 results	3	Automated systems = 2 result Manual method = 1 result
May 2021	22	Automated systems = 11 results Manual method = 11 results	2	Automated systems = 1 result Manual method = 1 results
September 2021	29	Automated systems = 8 results Manual method = 21 results	-	-
November 2021	22	Automated systems = 12 results Manual method = 10 results	-	-
March 2022	15	Automated systems = 5 results Manual method = 10 results	-	-
May 2022	22	Automated systems = 12 results Manual method = 10 results	3	Automated systems = 1 result Manual method = 2 results
September 2022	33	Automated systems = 19 results Manual method = 14 results	5	Manual method = 5 results
November 2022	19	Automated systems = 7 results Manual method = 12 results	-	-

mated systems and 13 were obtained using manual methods (Table 14).

When comparing the number of results obtained through manual methods and automated equipment, there is not a significant difference between the two, regardless of whether the results are satisfactory or unsatisfactory. Most of the unsatisfactory results were obtained by laboratories that used manual methods.

Conclusions

According to our study, a large proportion of the bacterial species identification results in the bacteriology EQC rounds were evaluated as satisfactory, with a percentage of 100% satisfactory results.

Only three EQC rounds had percentages of satisfactory results higher than 90.00%: *S. aureus* in the bacteriological examination of blood culture (90.91%) and *A. baumannii* in the bacteriological examination of purulent secretion (93.55% and 98.51%).

Regarding the techniques utilized by the Romanian Public Health Units for the bacteriology EQC programs, the manual methods are still widely used in many laboratories, both for the bacterial species identification and for antimicrobial susceptibility testing.

For *A. baumannii* susceptibility testing there was no EQC round with 100.00% satisfactory results recorded. On the contrary, *S. marcescens* susceptibility testing concluded with only 100.00% satisfactory results each time.

P. aeruginosa susceptibility testing showed the lowest percentages of satisfactory results (60.00% and 63.41%) for ceftriaxone. One possible reason for this may be the insufficient knowledge regarding intrinsic resistance of this microorganism, given the fact that *P. aeruginosa* is naturally resistant to ceftriaxone.

The findings of the intrinsic resistance part of the research indicated that the intrinsic resistance may not be as familiar as it should be for the laboratory personnel, and they do not consult their susceptibility testing results with specialized standards. As a result, there is a risk of misinterpreting susceptibility results, leading to inappropriate antimicrobial therapy.

Analyzing the bacteriology - screening for the detection of multidrug-resistant bacteria EQC program we observed that medical laboratories managed to provide accurate results.

However, the EQC rounds with *vancomycin – resistant Enterococcus faecalis* posed challenges for the participating laboratories, as these are the only rounds with no cases of 100.00% satisfactory results recorded.

Acknowledgments:

standard applicable to the accreditation of Proficiency Testing Schemes providers regarding the confidentiality of data provided by medical laboratories participating in External Quality Control programs.

References

1. G. A. POPESCU, R. ȘERBAN, A. NICULCEA, M. LEUȘTEAN, A. PISTOL, Consumul de antibiotice, Rezistența Microbiană și Infecții Asociate Asistenței Medicale în România, 2020, pp. 4 – 8;

2. G. A. POPESCU, I. CODIȚĂ, E. SZEKELY, R. ȘERBAN, G. RUJA, D. TĂLĂPAN, Ghid privind Enterobacteriaceae producătoare de carbapenemaze: diagnosticul, prevenirea transmiterii interumane și tratamentul infecțiilor produse, 2016, pp. 2-3;

3. I. JURADO-MARTÍN, M. SAINZ-MEJÍAS, S. MC-CLEAN, *Pseudomonas aeruginosa*: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors, International Journal of Molecular Science, 2021, (22) 3128, pp. 1;

4. E. N. TCHOUAKET, K. KRUGLOVA, I. BEOGO, D. SIA, S. ROBINS, E. BÉLANGER, M. JUBINVILLE, C. SÉGUIN, K. KILPATRICK, S. BOIVIN, J. LÉ-TOURNEAU, Economic evaluation of healthcare-associated infection prevention and control in long-term care: a systematic review protocol, Systematic Reviews, 2022, (11):261, pp. 2 – 3;

5. L. GIUBELAN, L. DRAGONU, A. C. STOIAN, F. DUMITRESCU, Profilul de rezistență la antimicrobiene al tulpinilor de *Klebsiella pneumoniae* într-o clinică de boli infecțioase, Romanian Journal of Infectious Diseases, 2021, 24(1), pp. 38 – 39;

6. E. MACHADO, P. COSTA, A. CARVALHO, Occurrence of Healthcare-Associated Infections (HAIs) by *Escherichia coli* and *Klebsiella spp*. Producing Extended-Spectrum β-lactamases (ESBL) and/or Carbapenemases in Portuguese Long-Term Care Facilities, Pathogens, 2022 (11) 1019, pp. 1 – 2;

7. R. ZIVKOVIC ZARIC, M. ZARIC, M. SEKULIC, N. ZORNIC, J. NESIC, V. ROSIC, T. VULOVIC, M. SPA-SIC, M. VULETA, J. JOVANOVIC, D. JOVANOVIC, S. JAKOVLJEVIC, P. CANOVIC. Antimicrobial Treatment of *Serratia marcescens* Invasive Infections: Systematic Review, Antibiotics, 2022 (11) 1228, pp. 1 – 2;

8. A. JANJUSEVIC, I. CIRKOVIC, R. MINIC, G. STE-VANOVIC, I. SOLDATOVIC, B. MIHALJEVIC, A. VIDOVIC, L. MARKOVIC DENIC, Predictors of *Vancomycin-Resistant Enterococcus spp.* Intestinal Carriage among High-Risk Patients in University Hospitals in Serbia, Antibiotics, 2022, (11) 1228, pp. 1 – 2;

9. F. TUTULESCU, G. D. MOGOSANU, A. BITA, L. E. BEJENARU, C. BEJENARU, G. RAU, C. N. OAN-CEA, R. CAPRUCIU, J. NEAMTU, Effect of boric acid and calcium ascorbatoborate esters against *methicillin-resistant Staphylococcus aureus*, Romanian Biotechnological Letters, 2018, Vol. 23, No. 6, pp. 14212 – 14213.