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PUBLIC HEALTH PROBLEM OF RESISTANT BACTERIA IN LOW AND MIDDLE-INCOME COUNTRIES, FOLLOWING THE EXAMPLE OF MOLDOVA

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Keywords: *antimicrobial resistance, bacteria, LMICs, susceptibility testing, bacteriophages, water treatment.*

Introduction. *Antimicrobial resistance is an important public health concern. This phenomenon has become an environmental problem, due to the spread of resistant microorganisms in water. This problem is now more visible in Low-and Middle-Income Countries, where it increases the social burden. One of the newest methods to fight antimicrobial-resistant bacteria is the use of strain-specific bacteriophages.* ***Material and methods.*** *The bacterial strains were obtained from inpatients and identified using VITEK 2 Compact system and culture. The disk diffusion method was used to determine the resistance profiles, which were then analyzed using EUCAST methodology. The presence of resistance mechanisms was checked by phenotypic testing. For research purposes, 31 bacterial strains were selected.* ***Results.*** *The strains of K. pneumoniae, P. aeruginosa, Acinetobacter spp., S. aureus, E. coli, and Enterococcus spp. were identified. The resistance profile of the isolates revealed: 61.5% of K. pneumoniae isolates were pan-drug-resistant, while 23,1% were only susceptible to Carbapenems. E. coli strains were extensively drug-resistant, 71.4% of P. aeruginosa and 75% of Acinetobacter spp. were pan-drug-resistant bacteria. The susceptibility profile of S. aureus strains showed that 3/4 were resistant to Cephalosporins and Fluoroquinolones.* ***Conclusions.*** *The study identified all six highly virulent and antibiotic-resistant bacterial pathogens in low and middle-income countries and Moldovan hospitals. The analysis conducted in the study could serve as an argument for using bacteriophages in water treatment as a cost-effective method to combat antimicrobial resistance.*

Cuvinte-cheie: *rezistența la antimicrobiene, bacterii, țări cu venituri mici și medii, teste de sensibilitate, bacteriofagi, tratarea apei.*

PROBLEMA BACTERIILOR REZISTENTE PENTRU SĂNĂTATEA PUBLICĂ ÎN ȚĂRILE CU VENITURI MICI ȘI MEDII DUPĂ EXEMPLUL MOLDOVEI

Introducere. *Rezistența la antimicrobiene reprezintă un subiect important pentru sănătatea publică. Fenomenul în cauză a devenit o problemă de mediu, fiind cauzat de răspândirea microorganismelor rezistente în apă. Aces fapt este mai vizibil în țările cu venituri mici și mijlocii, unde crește povara socială. Una dintre cele mai noi metode de combatere a bacteriilor rezistente la antimicrobiene este utilizarea bacteriofagilor specifici tulpinii.* ***Material și metode.*** *Tulpinile de bacterii au fost obținute de la pacienții internați și au fost identificate cu ajutorul sistemului VITEK 2 Compact și prin metoda clasică. Metoda discdifuzimetrică a fost aplicată pentru a determina profilurile de rezistență, care au fost apoi analizate folosind metodologia EUCAST. Prezența mecanismelor de rezistență a fost verificată prin teste fenotipice. Pentru cercetare au fost selectate 31 de tulpini bacteriene.* ***Rezultate.*** *Au fost identificate tulpinile de K. pneumoniae, P. aeruginosa, Acinetobacter spp., S. aureus, E. coli și Enterococcus spp. Profilul de rezistență al izolatelor a relevat: 61,5% din izolatele de K. pneumoniae au fost pan-rezistente, iar 23,1% au fost sensibile doar la carbapeneme. Tulpinile de E. coli au demonstrat reziztență extinsă, 71,4% din P. aeruginosa și 75% din Acinetobacter spp. erau bacterii pan-rezizstente. Profilul de sensibilitate al tulpinilor de S. aureus a arătat că 3/4 erau rezistente la cefalosporine și fluorochinolone.* ***Concluzii.*** *Studiul a identificat toți cei șase agenți patogeni bacterieni, extrem de virulenți și rezistenți la antibiotice, în țările cu venituri mici și medii și în spitalele din Republica Moldova. Analiza efectuată în cadrul studiului poate servi drept argument pentru utilizarea bacteriofagilor în tratarea apei ca metodă rentabilă de combatere a rezistenței la antimicrobiene.*

ABBREVIATIONS: *AMR* – Antimicrobial Resistance, *ARB* – Antimicrobial Resistant bacteria, *ESBL* – extended-spectrum beta-lactamases, *EUCAST* – European Committee on Antimicrobial Susceptibility Testing, *LMICs* – low- and middle-income countries, *MDR* – multidrug resistant, *PDR* – pan-drug-resistant, *WHO* – World Health Organization *XDR* – extensively drug resistant.

INTRODUCTION

In 1928, when Alexander Fleming discovered Penicillin – "the saving drug of the 20th century" – the glorious history of medicine began. Over the subsequent 60 years, the 13 classes of antibiotics that we still use to treat bacterial infections were discovered (1). Considering that the last 40 years have seen a multitude of epidemics and pandemics in which strictly human bacterial pathogens caused 44% of cases, contemporary medicine can only keep pace by using antimicrobials in treatment and prophylaxis, involving pre- and post-surgery antimicrobial and post-chemotherapy prophylaxis (2, 3, 4). Antibiotics have so far saved thousands of lives worldwide, but according to the laws of nature and ecosystems – everything must be in balance and living organisms must be constantly evolving. Thus, as early as 1940, the first enzyme that allowed *E. coli* strains to destroy penicillin was discovered (1, 5, 6). Since then, the phenomenon of antimicrobial resistance (AMR) has gained momentum, becoming a significant public health problem (5, 6, 7).

It should be noted that antimicrobial-resistant pathogens not only cause an increased number of deaths (mortality caused by multi-drug resistant (MDR) *P. aeruginosa* reaches up to 61% of cases, and pan-drug resistant (PDR) *K. pneumoniae* – maximum 71%) and disability, but also additional costs for hospitalization, treatment, and recovery, which cannot be accurately calculated (5, 8, 9). Specialists from various countries have concluded that the economic status of a country significantly influences the impact of AMR on the population, largely due to investment size in surveillance systems for antimicrobial resistance of microorganisms, but also to the presence and quality of alternative resources that can be used to fight infections (10). Some studies also list the private healthcare system as a determinant of AMR, citing the patient's substantial influence on antibiotic prescriptions due to commercial motivations (11).

Moreover, it is necessary to realize that humans are part of the ecosystem and are constantly influenced by other components of the ecosystem, especially as anthropogenic influence on the environment is unquestionable. It cannot be denied that people use chemicals, including antibiotics, in agriculture, fish farming, and animal husbandry to obtain richer harvests or better food-producing animals to compete on the market (12, 13, 14). Farms and animal husbandry are important sources of antimicrobial-resistant pathogens and an important component that ensures the continuous AMR cycle in the environment, due to animal care and handling processes including treatment, hygiene, and slaughter (14 - 17). However, by far the most important source of resistant microorganisms possessing genes for enzyme production (ESBLs – extended-spectrum beta-lactamases – or carbapenemases) is the sick human (whether or not admitted to a healthcare facility), whose contaminated biological products are discharged into water or released into the environment after minimal treatment, thus maintaining the AMR cycle (18 - 21). The limited number of methods used to combat AMR, combined with the preference of LMIC patients (and others) towards self-medication, are factors that make it difficult to align with the 2015 World Health Assembly Global Action Plan on AMR goals, particularly goals 4 and 5 (2, 3, 22, 23, 37). The overuse of antibiotics in LMICs, which has increased by 65% over the last decade, resulting in the emergence of multi-drug resistant (MDR) and extensively drug-resistant (XDR) superbugs, calls for innovative measures to combat AMR at the level of every component of the ecosystem (5, 7, 14, 19, 24). The European Union also emphasizes the role of Gram-negative bacteria in the etiology of infections with antimicrobial-resistant bacteria, which supports the results of World Health Organization (WHO) reviews concerning AMR (25). New antimicrobials and combinations are being sought and developed to combat MDR *Enterobacteriaceae* (e.g. Meropenem-Vaborbactam), but a cost-effective and less time consuming measure would be the use of bacteriophages on their own or in combination with usual antibiotics for per-

sonalized patient treatment, wastewater decontamination, farm animals, and crop plants (5, 8, 13, 23).

This article aims to identify and characterize potential targets for phages in bacteria circulating in the environment, originating from patients treated in medical institutions. These bacteria release biological fluids into the environment after minimal treatment. The focus lies on exploring the application of bacteriophages in LMICs as a cost-effective alternative to antibiotics and a method for water treatment, as they are readily available in nature and capable of development. This research gains significance, especially after WHO listed pathogens in 2017 that urgently demand new antibiotics to combat infections. Notably, ESKAPE pathogens account for over 70% of deaths attributed to antimicrobial resistance (AMR) (17, 26).

MATERIAL AND METHODS

Obtaining isolates

The isolates under investigation were sourced from patients admitted to *Timofei Mosneaga* Republican Clinical Hospital in Chisinau, Republic of Moldova. Substrates were collected only after meeting specific criteria: (I) the patient was over 18 years of age; (II) the patient consented to the use of the biological material for research; (III) the clinical picture of bacterial infection was established, and (IV) patients were admitted during the second and third quarters of 2022. Out of the total of isolates obtained, 31 strains met 4 criteria: (a) sourced from patients, (b) identifiable, (c) exhibited multi-resistance to antimicrobials, and (d) showed suspicion of a resistance mechanism. These were randomly selected and are described within this study.

Strain identification

The classical method – culture – was used to purify the isolates. Identification down to genus and species was done using the automated method – Vitek 2 Compact (BioMérieux, France). The standard protocol followed these steps: (I) Preparation of a bacterial suspension in sterile 0.45% NaCl saline solution using 18-24-hour fresh cultures in 3 ml polystyrene tubes; (II) Ensuring the suspension's turbidity was approximately 0.5 McFarland (±0.05 McFarland) with DensiCheck; (III) Bringing the ID cards to room temperature and placing them in transfer tube cassettes, which were then placed in the bacterial suspension tubes; (IV) Placing the cards within the cassette in a vacuum and initiating the card filling cycle; (V) Transferring the filled card cassette to the analyzer to obtain results after several hours.

Determination of isolate susceptibility

The resistance profile of the strains was determined using the disk diffusion method, with result interpretation following the EUCAST ver. 2022 standard. Mueller-Hinton solid medium and inoculum from a fresh 24h±6 h culture with a turbidity of 0.5 McFarland were used for this test. The procedure entailed: (a) Preparing the inoculum using sterile NaCl saline in 3 ml tubes and 3-5 colonies from a fresh culture; (b) Inoculating Petri dishes with Mueller-Hinton Agar medium using a swab; (c) Placing antibiotic-impregnated discs on the Petri dishes based on the species; (d) Incubating the Petri dishes with antibiograms at 37°C±1°C; (e) Reading and interpreting the results the following day according to EUCAST 2022 standards.

Determination of resistance mechanisms occurrence

Screening tests for ESBL, double diffusion test (double disc method), Combo test, and phenotypic tests were used to detect ESBL enzymes: class A – KPC, class B – MBL (VIM, NDM, IMP), class C – AmpC, class D – OXA-48, OXA-23. Tests for other resistance mechanisms were also used: colorimetric tests for the detection of carbapenemases – PACE Normand Poirel; immunochromatographic tests for detection of enzymes such as OXA-23 – *Acinetobacter* spp., OXA-48, and MBL for enterobacteria and *Pseudomonas aeruginosa*.

Statistical analysis

The proportion of strains among the total number of isolates, the prevalence of resistant bacterial strains within specific species, and the proportion of strains exhibiting phenotypically expressed resistance mechanisms were determined using the relative statistical indicator – proportion. These calculations were performed following the formulas:

$$\frac{No.of\ strains\ of\ particular\ species}{Total\ no.of\ bacterial\ strains} \text{x}100\ \% \qquad \text{(a)}$$

$$\frac{No.of\ bacterial\ strains\ of\ the\ species\ \ resistant\ to\ Antibiotic\ clas}{Total\ no.of\ strains\ of\ the\ species} \text{ x } 100\% \quad \text{(b)}$$

$$\frac{No.of\ bacterial\ strains\ of\ the\ \ species\ exposing\ a\ resistance\ mechanism}{Total\ number\ of\ strains\ of\ the\ species} \text{ x } 100\ \% \quad \text{(c)}$$

Bibliosemantic method

An online search was conducted in the PubMed and SCOPUS databases using the keywords: *antimicrobial resistance, bacteria, LMICs, susceptibility testing, bacteriophages, and water treatment.* This search yielded a total of 660 papers, from which 215 duplicates were removed. Articles that did not meet the specified criteria were also excluded:

- Written before 2012 (105 articles were excluded)
- Written in English (60 articles were excluded)
- Referring to ESKAPE pathogens in human health (65 articles were excluded)
- Not open access source (79 articles excluded)
- Not full-text type (99 papers excluded)

The remaining papers (n=37) were used as sources for the Introduction and the Discussion sections of this paper. Priority was given to articles detailing the status of ESKAPE pathogens in other LMICs globally, as well as in the three largest economies from distinct regions of the world: Japan (Asia), Germany (Europe), and the USA (Americas), for comparative analysis.

RESULTS

In the process of selecting strains for research, different biosubstrates were sampled. The proportion of biosubstrates was as follows: 8 isolates from blood and urine, 4 samples from pharyngeal swabs and wound content. Six samples were obtained from different biosubstrates: sputum, bronchoalveolar lavage, and feces. The isolated species were: *E. coli, K. pneumoniae, S. aureus, E. faecium, P. aeruginosa,* and *Acinetobacter* spp. (fig.1).

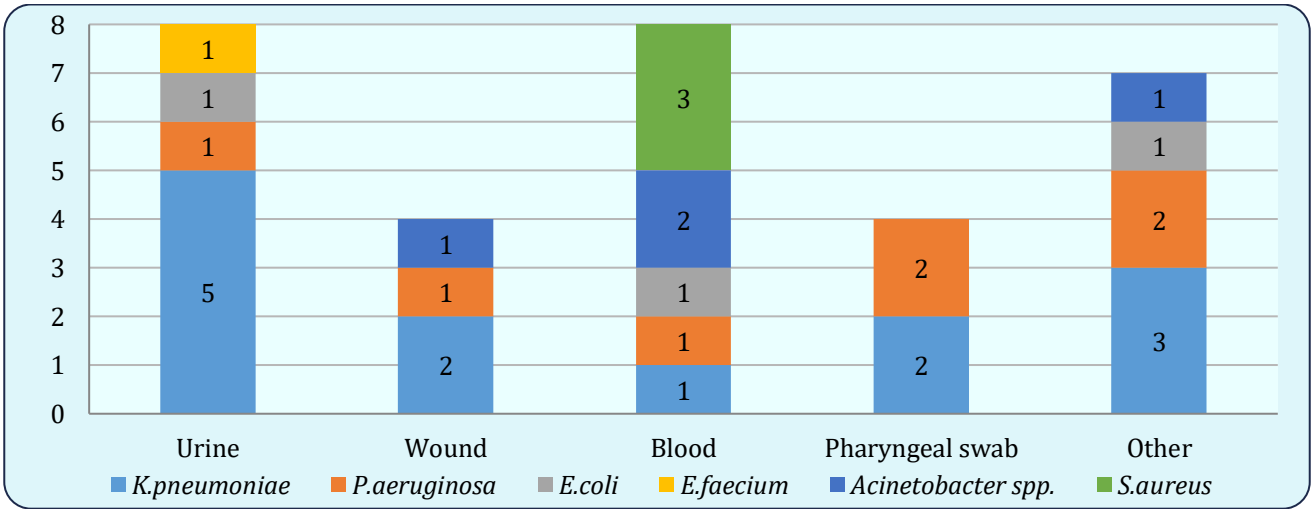

Figure 1. Biosubstrates investigated and bacterial species identified in each biosubstrate, absolute numbers.

Out of the 31 strains selected for research, 41.93% were *K. pneumoniae,* 22.58% were identified as *P. aeruginosa*, followed by *E. coli* and *Acinetobacter* spp. with 9.68% and 12.9%, respectively. *S. aureus* and *Enterococcus* spp. were identified in 9.68% and 3.23% of cases, respectively. Subsequently, the resistance profile for each isolate was determined and then grouped by species. Thus, 8 out of 13 isolates (61.5%) of *K. pneumoniae* were found to be PDR micro-organisms (non-susceptible to all commercially available antimicrobial agents), and 3 others (23.07%) were XDR strains, being susceptible only to Carbapenems. *E. coli* isolates (n=3) showed distinct

susceptibility patterns with selective susceptibility to agents of each class. It should be noted that for ord. Enterobacterales susceptibility to Penicillins, Cephalosporins, Carbapenems, Fluoroquin olones, and Aminoglycosides was tested, with a total of 16 antibiotics. Susceptibility testing of *P. aeruginosa* isolates included the same 5 classes of antibiotics (7 antibiotics) and the results are included in Table 1. The antibiotics tested from each class were: Piperacillin-tazobactam from Penicillins; Ceftazidime and Cefepime from Cephalosporins; Imipenem and Meropenem from Carbapenems; Ciprofloxacin from Fluoroquinolones and Amikacin from Aminoglycosides, respectively.

Table 1. Results of susceptibility testing of *P. aeruginosa* isolates.

Isolate	Penicillins	Cephalosporins	Aminoglycosides	Fluoroquinolones	Carbapenems
Isolate no. 13	R	R	R	R	R
Isolate no. 16	R	R	R	R	R
Isolate no. 17	R	I	R	R	I
Isolate no. 18	I	I	S	I	I
Isolate no. 22	R	R	R	R	R
Isolate no. 25	R	R	R	R	R
Isolate no. 29	R	R	R	R	R

Note: R – resistant; S – susceptible; I – intermediate (susceptible, increased exposure)

*The interpretations in the table mean that the results obtained were the same for all tested antimicrobials belonging to the same class.

The statistics showed that 71.4% of the isolates were PDR strains and one isolate (14.28%) showed characteristics of an XDR strain. Of all isolates, only one was susceptible to aminoglycoside antibiotics.

The 4 isolates identified as *Acinetobacter* spp. were tested for susceptibility to 6 antimicrobials categorized into 3 groups: Aminoglycosides, Fluoroquinolones, and Carbapenems. The results showed that 3 out of 4 isolates are PDR strains, and the fourth is susceptible only to Aminoglycosides (Tobramycin, Gentamycin, and Amikacin). The same testing procedure was done for *S. aureus* strains (tab. 2). Susceptibility of *S. aureus* strains was tested for: Cefoxitin (Cephalosporins), Ciprofloxacin, Ofloxacin, Norfloxacin (Fluroquinolone); Vancomycin (Glycopeptides and lipoglycopeptides), and Linezolid (Oxazolidinones).

Table 2. Resistance profile of *S. aureus* strains, n=4.

Isolate	Cephalosporins	Fluoroquinolones	Glycopeptides	Oxazolidinones
Isolate no. 8	R	I	S	S
Isolate no.18	R	R	S	S
Isolate no.19	R	R	S	S
Isolate no.30	R	R	S	S

Note: R – resistant; S – susceptible; I – intermediate (susceptible, increased exposure)

*The interpretations in the table mean that the results obtained were the same for all tested antimicrobials belonging to the same class.

The resistance profile of *S. aureus* strains showed that they were 100% susceptible to Glycopeptides and Oxazolidones. They were also 100% resistant to Cephalosporins and 3 out of 4 isolates were also resistant to Fluoroquinolones. The only isolate of the genus Enterococcus (*E. faecium*) was susceptible to Vancomycin and Linezolid and was resistant to Ampicillin.

Testing for the presence of resistance mechanisms revealed that among the 13 *K. pneumoniae* isolates, one exhibited the ESBL mechanism, while eight showed the presence of carbapene-

mases. In all cases, the OXA-48 type enzyme (subtype NDM) was detected in 62.5% of these instances (confirmed by immunochromatographic tests). In the other cases, although initially the strains were suspected of resistance mechanisms, this was not confirmed phenotypically. In the case of *E. coli,* 2 out of 3 isolates were ESBL-producing strains, the third being negative for both tested mechanisms. In the case of the 7 *P. aeruginosa* strains, testing for the presence of resistance mechanisms gave the following results: 71.42% (n=5) tested negative for carbapenemases, and in the case of the other 2 isolates, the result was positive, with one isolate producing NDM and the second, VIM enzymes. Finally, the double diffusion test and immunochromatographic tests of *Acinetobacter* spp. showed that 2 isolates (50%) were OXA-23 enzyme-producing strains, and the remaining were not.

DISCUSSIONS

Throughout our research on AMR, with a focus on infection etiology and the status of circulating strains (MDR, XDR, PDR), we have consistently found that antimicrobial resistance is a global problem, but the level of understanding and depth of approach varies from country to country and region to region. Consequently, we were able to compare our results with those obtained in individual country studies as well as regional and global studies. Inoue K. et al. concluded that mortality from infections with ESBL-producing microorganisms is higher compared to the rest of the ARB (22). The study by Silvester R. et al. showed that *K. pneumoniae* in all LMICs tends to exhibit ESBL resistance mechanisms, CP as well as combined genotypes, and these cause a greater burden on the healthcare system, especially as there are cases where healthy people are reservoirs of enzyme-producing bacteria, but also because they are the most frequently isolated bacteria encountered in hospital settings (10, 17, 20). Our study aligns with these findings, as Moldova is an LMIC, and AMR, as a multidimensional process, is a significant concern for the healthcare system.

However, many studies focus on various aspects of AMR occurrence and "frequency" in the health system, society, and the environment. Three studies conducted in Cameroon, Morocco, and Vietnam showed *Enterobacteriaceae* as the most frequently isolated microorganisms, followed by *S. aureus*, as we determined, but Camara N. and Haeeb A. determined an opposite situation in Tanzania and Saudi Arabia (2, 9, 27, 28, 29). When analyzing isolates according to the source (biosubstrate), studies focusing on uropathogens showed that *E. coli* is the most frequent uropathogen followed by *K. pneumoniae* (in Ethiopia and USA), compared to 2 studies from Romania showing 2 distinct situations - one study showed a similar situation as in Ethiopia, and the second one showed a distinct situation – with *P. aeruginosa* on the first place similar to the one in Mexico (14, 25, 30 31, 32). Still, none of them delivered results similar to the ones in this paper.

Finally, the comparative analysis of ESKAPE pathogen resistance profiles in different countries and regions is equally diverse. For *E. coli* isolates, studies in Tanzania showed the highest rate of resistance to Penicillins and Aminoglycosides, which correlates with the results of similar studies in Greece, Romania and Mexico and is similar to the results obtained in this paper (2, 31, 32, 33). However, there are also studies showing higher rates of resistance to other classes of antimicrobials – Fluoroquinolones and Cephalosporins (9, 32). Regarding *K. pneumoniae*, this study had similar results to those from Mexico and Tanzania, where the isolates had the second highest proportion of MDR profiles, and Morocco, where in addition to MDR status an alarming rate of carbapenemase-producing strains was detected (2, 29, 32). The results obtained on carbapenemase production by ord. Enterobacterales are in contrast to those obtained by researchers in Vietnam (LMIC) and Germany (9, 34). *P. aeruginosa*, another important microorganism in the etiology of infectious diseases, is one with a high proportion of PDR strains in this study, which is however very rarely found in other regions and countries, such as China, Greece, EU (33, 34, 35). The situation in *Acinetobacter* spp. is not much different in the same regions.

If we look at the Gram-negative microorganisms as a whole, part of the ESKAPE group, the trend of MDR strains (either XDR or PDR) circulation is constantly increasing, even reaching 100% in some LMICs. This phenomenon will inevitably create huge treatment costs, potentially amounting to tens of millions of dollars, as estimated by Australian epidemiologists (26, 29, 32, 36), calculated for a one-year period in Australia. LMICs do not have sufficient financial resources to treat pa-

tients and combat the long-term effects of reduced work capacities of the ill population, as well as the environmental damage and impacts on other economic sectors. To this end, both our study and the analyzed research focused on the description of pathogens of the ESKAPE group, the latter being potential targets for treatment with specific bacteriophages after their unintentional discharge into surface waters or sewage systems. Given the ubiquitous presence of bacteriophages in the environment and their ease of development, their use is much more economically efficient for water treatment and for slowing down the spread of AMR in the environment, and consequently, for minimizing the danger that this phenomenon poses for the human population.

CONCLUSIONS

1. AMR is an ongoing and significant issue that can lead to serious consequences. The data and analysis from this study demonstrate that AMR is a prevalent concern in healthcare institutions, irrespective of regional variations and circumstances.
2. To comprehend the AMR characteristics within the country, it is imperative to conduct an initial analysis on a small scale. This will facilitate the development of a comprehensive overview that can be compared with the regional situation.
3. This study revealed the concerning presence of AMR among ESKAPE pathogens in Moldovan hospitals, with limited treatment options available. This necessitates an urgent response and the exploration of innovative solutions.
4. This study stands as a cornerstone for the development of optimal solutions to combat AMR in the country, as it includes the analysis of potential cost-effective treatment targets.
5. Given the country's economic conditions, research and interventions aimed at characterizing the AMR phenomenon and identifying/developing bacteriophage targets for water treatment will serve as a crucial starting point to mitigate the impact of AMR. This is particularly significant considering that phages are present in the environment where resistant bacteria from hospitals ultimately contribute to the cycle of the AMR phenomenon.

CONFLICT OF INTEREST

The authors declare no conflict of interest in relation to the study and this paper.

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ETHICAL APPROVAL

The research protocol was positively approved by: (i) the Research Ethics Committee of *Nicolae Testemițanu* State University of Medicine and Pharmacy no. 7 issued on 09.01.2022 and (ii) the National Committee for Clinical Trial Ethics of the Ministry of Health of Republic of Moldova No. 1245 issued on 26.01.2022. The informed consent of every patient was obtained prior to sample collection procedure.

REFERENCES

1. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, Dhama K, Ripon MKH, Gajdács M, Sahibzada MUK, Hossain MJ, Koirala N. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. *J Infect Public Health*. 2021;14(12):1750-1766. doi:10.1016/j.jiph.2021.10.020
2. Camara N, Moremi N, Mghamba J, Eliakimu E, Shumba E, Ondoa P, Egyir B. Surveillance of antimicrobial resistance in human health in Tanzania: 2016-2021. *Afr J Lab Med*. 2023;12(1):2053. doi:10.4102/ajlm.v12i1.2053
3. Guo H, Hildon ZJ-L, Chow A. "Antibiotics are for everyone, our past and our future gene-rations, right? If antibiotics are dead, we will be in big trouble": Building on community values for public engagement on appropriate use of antibiotics in Singapore. *Front. Public Health*. 2022;10:1001282. doi:10.3389/fpubh.2022.1001282

4. Smith KF, Goldberg M, Rosenthal S, Carlson L, Chen J, Chen C, Ramachandran S. Global rise in humaninfectious disease outbreaks. *J. R. Soc.* 2014. doi:10.1098/rsif.2014.0950
5. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, et al. Antibiotic resistance: a rundown of a global crisis. *Infect Drug Resist*. 2018;11:1645-1658. doi:10.2147/IDR.S173867
6. Francine P. Systems Biology: New Insight into Antibiotic Resistance. *Microorganisms.* 2022; 10(12):2362. doi:10.3390/microorganisms10122362
7. Painter C, Faradiba D, Chavarina KK, Sari EN, Teerawattananon Y, Aluzaite K, Ananthakrishnan A. A systematic literature review of economic evaluation studies of interventions impacting antimicrobial resistance. *Antimicrob Resist Infect Control*. 2023;12(1):69. doi:10.1186/s13756-023-01265-5
8. Ragupathi NKD, Muthuirulandi Sethuvel DP, Gopikrishnan M, Dwarakanathan HT, Murugan D, Biswas I, et al. Phage-based therapy against biofilm producers in gram-negative ESKAPE pathogens. *Microb Pathog.* 2023;178:106064. doi:10.1016/j.micpath.2023.106064
9. Van An N, Hoang LH, Le HHL, Thai Son N, Hong LT, Viet TT, et al. Distribution and Antibiotic Resistance Characteristics of Bacteria Isolated from Blood Culture in a Teaching Hospital in Vietnam During 2014-2021. *Infect Drug Resist*. 2023; 16:1677-1692. doi:10.2147/IDR.S402278
10. Silvester R, Madhavan A, Kokkat A, Parolla A, et al. Global surveillance of antimicrobial resistance and hypervirulence in Klebsiella pneumoniae from LMICs: An in-silico approach. *Sci Total Environ*. 2022;802:149859. doi:10.1016/j.scitotenv.2021.149859
11. Maugeri A, Barchitta M, Puglisi F, Agodi A. Socio-economic, governance and health indicators shaping antimicrobial resistance: an ecological analysis of 30 european countries. *Global Health*. 2023;19(1):12. doi:10.1186/s12992-023-00913-0
12. Goryluk-Salmonowicz A, Popowska M. Factors promoting and limiting antimicrobial resistance in the environment - Existing knowledge gaps. *Front Microbiol.* 2022; 13:992268. doi:10.3389/fmicb.2022.992268
13. Tsvetanova Z, Dimitrov D, Najdenski H. Prevalence of AntimicrobialResistance in a Bulgarian Drinking Water Supply System. *Water Supply*. 2022;22(9):7059. doi:10.2166/ws.2022.302
14. Woyda R, Oladeinde A, Abdo Z. Chicken Production and Human Clinical Escherichia coli Isolates Differ in Their Carriage of Antimicrobial Resistance and Virulence Factors. *Appl Environ Microbiol*. 2023;89(2): e0116722. doi:10.1128/aem.01167-22
15. Aslam B, Khurshid M, Arshad MI, Muzammil S, Rasool M, Yasmeen N, et al. Antibiotic Resistance: One Health One World Outlook. *Front. Cell. Infect. Microbiol*. 2021;11: 771510. doi:10.3389/fcimb.2021.771510
16. Olaru ID, Walther B, Schaumburg F. Zoonotic sources and the spread of antimicrobial resistance from the perspective of low and middle-income countries. *Infect Dis Poverty*. 2023; 12(1):59. doi:10.1186/s40249-023-01113-z
17. Perestrelo S, Amaro A, Brouwer MSM, Clemente L, Ribeiro Duarte AS, Kaesbohrer A, et al. Building an International One Health Strain Level Database to Characterise the Epidemiology of AMR Threats: ESBL-AmpC Producing E. coli as An Example-Challenges and Perspectives. *Antibiotics (Basel).* 2023;12(3):552. doi:10.3390/antibiotics12030552
18. Acolatse JEE, Portal EAR, Boostrom I, Akafity G, Dakroah MP, Chalker VJ, et al. Environmental surveillance of ESBL and carbapenemase-producing gram-negative bacteria in a Ghanaian Tertiary Hospital. *Antimicrob Resist Infect Control*. 2022;11(1): 49. doi:10.1186/s13756-022-01090-2
19. Kajova M, Khawaja T, Kantele A. European hospitals as source of multidrug-resistant bacteria: analysis of travellers screened in Finland after hospitalization abroad. *J Travel Med*. 2022; 29(4):taac022. doi:10.1093/jtm/taac022
20. Rolbiecki D, Korzeniewska E, Czatzkowska M, Harnisz M. The Impact of Chlorine Disin-fection of Hospital Wastewater on Clonal Similarity and ESBL-Production in Selected Bacteria of the Family Enterobacteriaceae. *International Journal of Environmental Research and Public Health*. 2022;19(21): 13868. doi:10.3390/ijerph192113868
21. Salazar C, Giménez M, Riera N, Parada A, Puig J, Galiana A, et al. Human microbiota drives hospital-associated antimicrobial resistance dissemination in the urban environment and mirrors patient case rates. *Microbiome*. 2022; 10(1):208. doi:10.1186/s40168-022-01407-8
22. Inoue K, Kobayashi S, Sato K, Kanno H, Kantou R, Naganuma Y, et al. Regional Antimicrobial Stewardship Program in a Provincial Medical Zone in Japan: a Multifaceted Approach. *Jpn J Infect Dis*. 2022;75(4):347-354. doi:10.7883/yoken.JJID.2021.577

23. Terreni M, Taccani M, Pregnolato M. New Antibiotics for Multidrug-Resistant Bacte-rial Strains: Latest Research Develop-ments and Future Perspectives. *Molecules.* 2021; 26(9):2671. doi:10.3390/molecules26092671
24. Mir MA, Nabi B, Ahlawat S, Kumawat M, Aisha S. Combating human bacterial infec-tions. *Human Pathogen Microbes*. 2022;71-102. doi:10.1016/B978-0-323-96127-1.00008-5
25. Rusu A, Tiliscan C, Adamescu AI, Ganea OA, Arama V, Arama SS. Carbapenemase-producing uropathogens in real life: epidemiology and treatment at a County Emergency Hospital from Eastern Romania. *J Med Life*. 2023;16(5):707-711. doi:10.25122/jml-2023-0139
26. Al Dabbagh M, Alghounaim M, Almaghrabi RH, Dbaibo G, Ghatasheh G, Ibrahim HM, et al. Narrative Review of Healthcare-Associated Gram-Negative Infections Among Pediatric Patients in Middle Eastern Countries. *Infect Dis Ther*. 2023;12(5):1217-1235. doi:10.1007/s40121-023-00799-w
27. Djuikoue CI, Yamdeu Djonkouh W, Epie Bekolo C, Kamga Wouambo R, Carrel Founou R, Djouela Djoulako PD, et al. Prevalence and Antibiotic Resistance Pattern of Streptococcus, Staphylococcus, Neisseria meningitidis and Enterobacteriaceae in Two Reference Hospitals of Yaoundé: An Overview before and during COVID-19 Pandemic Era. *Antibiotics (Basel).* 2023;12(5):929. doi:10.3390/antibiotics12050929
28. Haseeb A, Faidah HS, Algethamy M, Al ghamdi S, Alhazmi GA, Alshomrani AO, et al. Antimicrobial Usage and Resistance in Makkah Region Hospitals: A Regional Point Prevalence Survey of Public Hospitals. *International Journal of Environmental Research and Public Health*. 2022;19(1):254. doi:10.3390/ijerph19010254
29. Nejjari C, El Achhab Y, Benaouda A, Abdelfattah C. Antimicrobial resistance among GLASS pathogens in Morocco: an epidemiological scoping review. *BMC Infect Dis*. 2022;22(1):438. doi:10.1186/s12879-022-07412-4
30. Gebretensaie Y, Atnafu A, Girma S, Alemu Y, Desta K. Prevalence of Bacterial Urinary Tract Infection, Associated Risk Factors, and Antimicrobial Resistance Pattern in Addis Ababa, Ethiopia: A Cross-Sectional Study. *Infect Drug Resist*. 2023;16:3041-3050. doi:10.2147/IDR.S402279
31. Petca RC, Mareş C, Petca A, Negoiţă S, Popescu RI, Boţ M, et al. Spectrum and Antibiotic Resistance of Uropathogens in Romanian Females. *Antibiotics (Basel).* 2020; 9(8):472. doi:10.3390/antibiotics9080472
32. Uc-Cachón AH, Gracida-Osorno C, Luna-Chi IG, Jiménez-Guillermo JG, Molina-Salinas GM. High Prevalence of Antimicrobial Resistance Among Gram-Negative Isolated Bacilli in Intensive Care Units at a Tertiary-Care Hospital in Yucatán Mexico. *Medicina (Kaunas).* 2019;55(9):588. doi:10.3390/medicina55090588
33. Manolitsis I, Feretzakis G, Katsimperis S, Angelopoulos P, Loupelis E, Skarmoutsou N, et al. A 2-Year Audit on Antibiotic Resistance Patterns from a Urology Department in Greece. *Journal of Clinical Medicine*. 2023; 12(9):3180. doi:org/10.3390/jcm12093180
34. Rödenbeck M, Ayobami O, Eckmanns T, Pletz MW, Bleidorn J, Markwart R. Clinical epidemiology and case fatality due to antimicrobial resistance in Germany: a systematic review and meta-analysis, 1 January 2010 to 31 December 2021. *Euro Surveill*. 2023;28(20):2200672. doi:10.2807/1560-7917
35. Lyu J, Chen H, Bao J, Liu S, Chen Y, Cui X, et al. Clinical Distribution and Drug Resistance of Pseudomonas aeruginosa in Guangzhou, China from 2017 to 2021. *Journal of Clinical Medicine*. 2023;12(3):1189. doi:10.3390/jcm12031189
36. Wozniak TM, Dyda A, Merlo G, Hall L. Disease burden, associated mortality and economic impact of antimicrobial resistant infections in Australia. *Lancet Reg Health West Pac*. 2022; 27:100521. doi:10.1016/j.lanwpc.2022.100521
37. World Health Assembly. Global Action Plan on Antimicrobial Resistance. 2015. Avai-lable online: https://www.emro. who.int/ health-topics/drug-resistance/ global-action-plan.html [Accessed on 29 Dec.2023].

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