https://doi.org/10.54361/ajmas.269120

Original article

Antibiotic Resistance Patterns in Clinical Bacterial Isolates from Gharyan City, Libya

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Abstract
Antimicrobial agents are widely used to treat bacterial infections; however, their misuse contributes to the growing problem of antibiotic resistance. This study assessed the prevalence of antibiotic resistance among bacterial isolates from patients in Gharyan City, Libya, and explored behavioral factors influencing resistance. Between January and June 2024, 66 clinical samples (urine, stool, vaginal swabs, blood, pus, and sputum) were collected from seven medical laboratories. A questionnaire survey was conducted among three groups: medical workers (n=50), non-medical individuals (n=50), and pharmacists (n=50). Laboratory identification employed selective media, Gram staining, and microscopy, while antibiotic susceptibility was tested using disc diffusion. Results revealed widespread inappropriate antibiotic use: 70% of pharmacists dispensed antibiotics without prescriptions, 40% provided incorrect dosages, and only 10% held formal pharmacy qualifications. Among medical workers, 40% used antibiotics without prescriptions and 56% failed to complete treatment courses. Infections were more common in females (77%) than males (23%), with the highest prevalence in younger age groups (<32 years, 39.4%). Staphylococcus spp (32%) and E. coli (27%) were the most frequent isolates. Overall resistance was high (75%), particularly in Pseudomonas spp (100%), E. coli (83%), and Staphylococcus spp (76%). Azithromycin (67%) and Doxycycline (49%) showed the highest resistance rates, while Meropenem (2%) and Nalidixic acid (3%) remained largely effective. These findings highlight alarming levels of antibiotic resistance in Gharyan, driven by misuse and poor prescribing practices. Strengthening pharmacist and physician training, enforcing prescription regulations, and raising public awareness are essential to mitigate resistance and preserve antibiotic efficacy.
Keywords. Antibiotic Resistance, Bacterial Infection, Misuse of Antibiotics, Control and Surveillance.

Introduction
Antimicrobial resistance (AMR) refers to the capability of certain microorganisms to withstand the effects of medications that were previously effective in treating infections caused by them. This resistance allows these pathogens to survive and multiply even in the presence of these drugs, ultimately leading to ineffective treatments and posing a significant healthcare challenge [1]. Each time an antimicrobial drug is administered, it loses effectiveness for everyone, as increased usage heightens the risk of bacteria developing resistance. Antibiotics work by either killing bacteria or inhibiting their growth, making them essential in treating bacterial infections. In veterinary medicine, these drugs are commonly utilized to address and prevent diseases in animals, as well as to control the transmission of infections. The rise of antimicrobial-resistant organisms poses a significant global health threat, currently causing approximately 700,000 deaths each year, with projections suggesting this figure could reach 10 million by 2050 [2].
AMR is a growing concern, especially in developing countries, and is expected to increase in the coming years due to higher antimicrobial use in public and veterinary care. Antimicrobials are used for livestock farming at a significantly higher rate (73–100%) than in human medicine, driven by a growing middle class's demand for animal-based protein [2]. Health facility-acquired infections are often caused by antibiotic-resistant organisms, particularly *Staphylococcus aureus and Gram-negative rods such as Escherichia coli* and *Pseudomonas aeruginosa*. In hospitals, high antibiotic use selects for these organisms, contributing to resistance. Bacteria develop resistance through various mechanisms, including enzyme inactivation (beta-lactamases), altered structural targets, reduced drug permeability, antibiotic export pumps, and altered metabolic pathways [1].
The overuse and misuse of antibiotics often lead to the development of antibiotic resistance in microorganisms. The abuse of these drugs over time has led to the emergence of resistant microbes in recent years; the healthcare sector is currently faced with a crowded market as it works hard to develop new medications while also overcoming difficulties in treatments brought on by the quickly increasing levels of drug resistance. Causes of antimicrobial resistance associated with human health are due to the unsuitable usage of antimicrobials in human medicine. The global issue of antibiotic resistance has been greatly exacerbated by the overuse and misuse of antibiotics [1,3].

Methods
Samples Collection
Between January 25th and June 17th, samples were gathered from seven laboratories in Gharyan. We utilized questionnaires to survey three different groups: individuals working in the medical field, those outside the medical field, and pharmacists. A total of 66 samples were collected, which included urine, stool, vaginal

***Received**: 10-11-2025 - **Accepted**: 09-01-2026 - **Published**: 15-01-2026*

swabs, pus, and sputum. Among the samples, 51 were from females and 15 from males. The age range of participants spanned from less than one year to 94 years.
Various techniques employed included the preparation of selective and differential media, such as CLED agar (Cysteine Lactose Electrolyte Deficient), MacConkey agar, and blood agar, which facilitated the growth of pathogenic bacteria. The Gram stain method, along with microscopic examination, is a common technique in medical microbiology laboratories for staining bacterial samples. It aids in identifying different types of bacteria by describing their shapes and arrangements.
Following the culturing and identification of the bacteria, we applied a suspension of the pathogenic bacteria onto Muller-Hinton agar (MHA) using a sterile swab. Standardized antibiotic disks were placed using forceps, and the plates were incubated at 37°C for 48 hours. The zones of inhibition were then measured with a ruler to evaluate antibiotic sensitivity, as detailed in a previous study (4), and the Clinical and Laboratory Standards Institute (CLSI). Limitations of the study were that all laboratories utilized standardized antibiotic disk reference values from UROCAST or CLSI. Instead, they rely on the diameter of the zone of inhibition (ZI) to categorize results as sensitive (S) or resistant (R). The highest sensitivity is indicated as (S++++), while lower sensitivity levels are represented as (S++).

Results

Table 1. evaluates the education of pharmacists with the population and antibiotics used. We show that (70%) of pharmacists give antibiotics without a prescription, and (30%) do not explain the activation of antibiotics. (40%) gives a false concentration of antibiotics. This table indicates that we need experience.

Table 1. Questionnaire of pharmacists

Questions	Yes %	No. %	Sometimes %
Do you describe antibiotics to patients?	07 (70%)	02 (20%)	01 (10%)
Do you give the patients a prescription for antibiotics?	10 (100%)	00 (0%)	00 (0%)
Do you explain to the patients the side effects of antibiotics?	06 (60%)	03 (30%)	01 (10%)
Do you explain to the patients how to take the antibiotics?	10 (100%)	00 (0%)	00 (0%)
Do you advise the patients to take antibiotics at a specific time and for the required period?	10 (100%)	00 (0%)	00 (0%)
Have you ever been prescribed medication for patients?	01 (10%)	05 (50%)	04 (40%)

Table 2 shows that most health workers are not pharmacists or do not study the pharmacology specialties. (70%) not a pharmacist, only (10%) were in pharmacy graduation, and (20%) were in the high institute of pharmacy.

Table 2. Academic qualifications of pharmacists in Gharyan

Qualification	No(%)
Pharmacy institute graduate	03(30%)
Bachelor of Pharmacy	01(10%)
Graduate of Medicine	01(10%)
Medicine student	02(20%)
Bachelor of Dentistry	01(10%)
Dentist student	02(20%)

Table 3 explaine misuse and incorrect procedure to take antibiotics between population in medlical field where (40%) of them take the treatments without prescription, (52%) the pharmacist give Antibiotics with out prescription, (56%) not complete the recommended rygemen period of antibiotics, (32%) they not take the medicine on correct time and (28%) take antibiotics with out medical reason.

Table 3. People from the medical field in Gharyan city

Questions	Yes %	No. %	Sometimes %
Do you take antibiotics when you are sick with a prescription and after visiting a doctor?	10 (40%)	10 (40%)	05 (20%)
Do you constantly take antibiotics?	00 (0%)	22 (88%)	03 (12%)
Do you suffer from a disease that requires taking antibiotics?	01 (4%)	18 (72%)	06 (24%)
Did the pharmacist sell you antibiotics without a prescription?	13 (52%)	11 (44%)	01 (4%)
Do you continue to take the antibiotics on time and for the required duration?	14 (56%)	08 (32%)	03 (12%)

Received: 10-11-2025 - *Accepted*: 09-01-2026 - *Published*: 15-01-2026

Do you stop taking antibiotics as soon as you get better without completing the course of antibiotics recommended by your doctor?	14 (56%)	06 (24%)	05 (20%)
Have you ever used antibiotics as a preventative measure to avoid getting sick?	07 (28%)	18 (72%)	00 (0%)
Did your doctor explain to you the correct use of antibiotics?	16 (64%)	08 (32%)	01 (4%)

Data in Table 4 showed the misuse of antibiotics among the population not working or studying in the medical field, to assess the education of the population. The answers were surprising because misuse was found, but with less prevalence than among the medical population. (12%) Take antibiotics without a prescription, (44%) pharmacists described the antibiotics without a medical description, (20%) did not finish the period of treatment, (16%) did not take the treatment at the correct time

Table 4. People from another field in Gharyan city

Questions	Yes	No	Sometimes
Do you take antibiotics when you are sick with a prescription and after visiting the doctor?	21 (84%)	03 (12%)	02 (8%)
Do you constantly take antibiotics?	03 (12%)	18 (72%)	04(16%)
Do you suffer from a disease that requires taking antibiotics?	05 (20%)	18 (72%)	02 (8%)
Did the pharmacist sell you antibiotics without a prescription?	11 (44%)	12 (48%)	02 (8%)
Do you continue to take the antibiotics on time and for the required duration?	19 (76%)	04 (16%)	02 (8%)
Do you stop taking antibiotics as soon as you get better without completing the course of antibiotics recommended by your doctor?	05 (20%)	14 (56%)	06 (24%)
Have you ever used antibiotics as a preventative measure to avoid getting sick?	03 (12%)	21 (84%)	01 (4%)
Did your doctor explain to you the correct use of antibiotics?	22 (88%)	01 (4%)	02 (8%)

Table 5 presented that the infected patients were 66 cases, the female number (51) by percent (77%) was more than the male number (15) by (23%).

Table 5. Prevalence of infection among genders

Gender	No(%)
Female	51(77%)
Male	15(23%)

The studied age in table 6 ranged from (< 1y-94y), the infection among age (< 1y-31y) with number 26 (39.4%), age (32y-67y) with 24 (36.4%), and age (68y-94y) with 16 (24.2%).

Table 6. Prevalence of infection among ages

Age	No(%)
< 01y-31y	26(39.4%)
32y-67y	24(36.4%)
68y-94y	16(24.2%)

Table 7 exhibited that most of the samples that came to laboratories were urin sample with 57 (86%), blood samples 02 (03%), stool samples 02(03%), pus samples 02(03%), vaginal swabs 02(03%), and sputum 01 (02%).

Table 7. Number of samples

samples	No(%)
Urine	57(86%)
Blood	02(03%)
Stool	02(03%)
Pus	02(03%)
Vaginal Swab	02(03%)
Sputum	01(02%)

***Received**: 10-11-2025 - **Accepted**: 09-01-2026 - **Published**: 15-01-2026*

Table 8 shows the isolated bacteria and the distribution of resistance (R) and sensitivity (S). *Staphylococcus spp* 21 (32%), *E. coli* 18 (27%), *Klebsiella spp* 16 (24%), *Streptococcus spp* 09 (14%), *Pseudomonas spp* 02(3%). The total of resistance is 50 (76%), and sensitive 16 (24%). *Pseudomonas spp* more resistance 02 (100%), *E. coli* 15(83%), *Staphylococcus spp* 16(76%), *Klebsiella spp* 11(69%), and *Streptococcus spp* 06 (67%).

Table 8. Distribution of bacteria in relation to Resistance

Microbes	No.	(%)	R	(%)	S	(%)
Staphylococcus spp	21	(32%)	16	(76%)	05	(24%)
E. coli	18	(27%)	15	(83%)	03	(17%)
Klebsiella spp	16	(24%)	11	(69%)	05	(31%)
Streptococcus spp	09	(14%)	06	(67%)	03	(33%)
Pseudomonas spp	02	(03%)	02	(100%)	00	(00%)

Table 9 concludes the percent resistance of antibiotics that are used in sensitivity tests. The most antibiotic that seems to be resistant is Azithromycin (67%). After that, Doxycycline (49%), Trimethoprim solution, Amikacin and Tetracycline (38%), Colistine and amoxicillin, and Erythromycin and Ceftoxitin (36%). Cefuroxime(32%). Ciprofloxacin (30%) and Nitrofuranation (29%) had less resistance included Ampicillin and Amoxicillin-Clavulanate AC, Tobramycin (18%). Vancomycin (15%), Cefotaxime, Trimethoprim+sulphamethoxazole (14%). Imipenem (12%), Novobiocin, and Ofloxacin (11%). The lower resistance is Streptomycin (06%), Naldexic acid (03%), and Meropenem (02%).

Table 9. The percentage of resistance against antibiotics

Antibiotic	No.	%	Antibiotic	No.	%	Antibiotic	No	%
Ciprofloxacine	20	30	Trimethoprime solution	25	38	Cefuroxime	21	32
Doxycycline	32	49	Cefotaxime	09	14	Nitrofuranation	19	29
Amoxicillin	24	36	Amikacin	25	38	Erythromycin	24	36
Colistine	24	36	Amoxicillin& Clauvulanice	12	18	Azithromycin	44	67
Imipenem	08	12	Ceftoxitin	24	36	Vancomycin	10	15
Ampicillin	12	18	Meropenem	01	02	Novobiocin	07	11
Naldexic acid	02	03	Tobramycin	12	18	Streptomycin	04	06
Ofloxacin	07	11	Trimethoprim+ sulphamethoxazole	09	14	Tetracycline	25	38

Discussion

In this study, we aimed to evaluate the prevalence of antibiotic misuse in Gharyan City, located in western Libya, and to assess the level of education and perceptions regarding antibiotic use among the population and pharmacists. The results revealed a high prevalence of overuse and inappropriate use of antibiotics among both medical professionals and the general population.

Among individuals working in the medical field, misuse was particularly evident: 40% reported taking antibiotics without a prescription, 52% obtained antibiotics directly from pharmacists without prescriptions, 56% did not complete the recommended treatment course, 32% failed to take the medication at the correct times, and 28% used antibiotics without medical justification. Misuse was also observed among the general population, though at lower rates: 12% took antibiotics without a prescription, 44% received antibiotics from pharmacists without proper documentation, 20% did not complete the treatment course, and 16% failed to take the medication at the correct times. Pharmacists demonstrated significant shortcomings in practice. Seventy percent dispensed antibiotics without prescriptions, 30% failed to provide adequate explanations regarding antibiotic use, and 40% offered incorrect concentrations. A review of pharmacist qualifications indicated that 70% lacked proper credentials; only 10% were pharmacy graduates, while 20% had attended the High Institute of Pharmacy. These findings highlight the urgent need for improved pharmacist education and stricter regulation to ensure safe and effective prescribing practices.

Our results suggest that antibiotic misuse is more prevalent among individuals in the medical field compared to the general population, posing a serious risk for the development of antibiotic resistance. A related study conducted in Zawia, Libya, focusing on university students, found that students demonstrated significant understanding and positive attitudes toward appropriate antibiotic use. These findings were compared with similar studies in Benghazi, Libya, as well as in Jordan, Europe, Nigeria, and other regions, where misuse appeared more prevalent. Researchers emphasized the importance of enhanced educational programs for students to address this issue [5]. Globally, studies in Southeast Asia have documented widespread self-medication with antibiotics, often without physician prescriptions, increasing the risk of improper use and the spread of drug-resistant microorganisms. Antimicrobial resistance (AMR) is influenced by multiple factors, including human practices (overuse, misuse, insufficient infection control, and lack of awareness), animal-related practices (extensive antibiotic use in livestock and aquaculture, transmission through food chains and direct contact), environmental factors (release of antibiotics and resistant bacteria into water,

***Received**: 10-11-2025 - **Accepted**: 09-01-2026 - **Published**: 15-01-2026*

soil, and waste systems), and wildlife-related factors (transmission through contact with wildlife and habitat encroachment) [3,6].

Excessive and unregulated use of antibiotics significantly contributes to the rise of drug resistance. Practices such as over-prescription and pressures to achieve patient outcomes promote the survival of resistant microorganisms while eliminating susceptible ones, thereby fostering AMR. Numerous studies have confirmed that antibiotic misuse is a key driver of AMR, leading to bacterial adaptations such as unnecessary use of broad-spectrum antibiotics, prolonged treatment courses, infections with multi-drug resistant (MDR) bacteria, and genetic mutations [2,7]. Increased antibiotic consumption typically correlates with higher resistance levels, whereas reducing usage can help mitigate this problem [2]. Moreover, antibiotic concentrations within the body can exacerbate resistance by encouraging genetic changes. The One Health approach underscores the importance of understanding the biological, environmental, and socioeconomic factors associated with the emergence and transmission of resistance, including microorganisms, vectors, host organisms, ecosystems, and cultural practices [3].

Both age and gender play crucial roles in relation to infections and are associated with various other factors like environment, health education, lifestyle, and the presence of chronic diseases. In our research, we found that females experienced a higher rate of infections compared to males. This could be attributed to the greater number of females in the sample, as well as the fact that urinary tract infections, which are more common in women, constituted a significant portion of the cases. Additionally, women tend to take more proactive steps regarding their health, often seeking medical advice more frequently than men [8]. Overall, numerous past studies have indicated that males tend to be more vulnerable to infections compared to females, attributed to several factors such as variations in the immune system, genetic makeup, hormonal differences, and physiological distinctions. Additionally, men often have more exposure to infection sources than women. The rate of infection can also be influenced by other elements, including the nature of the infection, the geographical area affected by outbreaks, and the availability and quality of healthcare services. Research has shown that women generally exhibit stronger immune responses (as noted in a study from Bangladesh by Sara *et al.*) [9]. Furthermore, occupational and recreational activities may impact exposure levels to pathogens, and in certain cultures, boys may be taken to healthcare facilities more frequently than girls by their parents [8, 10, 11]. Distribution of infection among age groups in our study showed that young people (<1y-31y) with (39.4%) and young old patients (32y-67y) with (36.4%) were more infected. This may be referring to the lower immunity of young cases, and they are more in contact with other people. This puts them at risk of infection more than other age groups. Older patients (68y - 94y) have a low number and are less infected with (24.2%) because the Libyan community is young and close to this age at death. To our knowledge, the increase in age causes a decline in the immune system, so the level of infection will increase. In addition to the interaction between the weekend of immunity and bacterial pathogenicity, the development of vaccines, novel antibiotic resistance, multidrug resistance, malnutrition, and other factors can improve outcomes for bacterial infections in the growing elderly population (8). Older adults tend to be more vulnerable to bacterial infections, including urinary tract infections caused by *E. coli, Klebsiella species*, and *Pseudomonas species*. They are also at risk for respiratory infections from *Streptococcus* and *Moraxella species*, as well as skin and soft tissue infections by *Staphylococcus* and *Pseudomonas species*, among others (8, 11-15). Our findings align with these observations, suggesting that infections occur across different age groups. Interestingly, our results indicate that elderly individuals appear less susceptible than younger ones. This may be attributed to their lower representation in the community of Libya and a smaller sample size in our study. Additionally, it's important to note that our research focused on outpatients, while many other studies are based in hospital settings.

Most of our samples are urine, and Gram-negative (G-Ve) bacteria are more distributed in urine (55%). *Staphylococcus spp* 32%. *E.coli* 27%. *Klebsiella spp.* 24%. *Streptococcus.spp* 14%. *Pseudomonas spp.* 02%. High resistance among isolated bacteria with percent (76%). Resistance is *to Pseudomonas. spp* (100%). *E.coli* (83%). *Staphylococcus. spp* (76%). *Klebsiella. spp and streptococcus. spp* (69%.67%). Our finding is in agreement with many other reports. A report from the antimicrobial resistance collaborators highlighted that in 2019, *E. coli* emerged as the primary cause of deaths linked to antimicrobial drug resistance, followed by *Staphylococcus aureus*. Other significant pathogens included *Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumannii,* and *Pseudomonas aeruginosa*. Collectively, these pathogens accounted for approximately 929,000 deaths directly attributed to antimicrobial resistance (AMR) worldwide, leading to an estimated total of 3.57 million deaths (ranging from 2.62 to 4.78 million) due to AMR.

Notably, the Western region of Sub-Saharan Africa experienced a higher toll, with around 27.3 deaths (approximately 100,000 individuals) per 100,000 people [2]. The most common antibiotics with resistance among bacterial infections were Azithromycin (67%). After that, Doxycycline (49%), Trimethoprim solution, Amikacin and Tetracycline (38%), Colistine and amoxicillin, and Erythromycin and Ceftoxitin (36%). Cefuroxime(32%). Ciprofloxacin (30%) and Nitrofuranation (29%). Less resistance included Ampicillin and Amoxicillin-Clavulanate AC, Tobramycin (18%). Vancomycin (15%), Cefotaxime, Trimethoprim+sulphamethoxazole (14%). Imipenem (12%), Novobiocin, and Ofloxacin (11%). The lowest resistance is Streptomycin (06%), Naldexic acid (03%), and Meropenem (02%). A study conducted in 2017 in Tripoli, Libya, revealed that the most frequently isolated bacteria from urine samples with positive culture

***Received**: 10-11-2025 - **Accepted**: 09-01-2026 - **Published**: 15-01-2026*

results were *Staphylococci spp.* (64.5%), *E. coli* (29.0%), and *Klebsiella pneumonia* (6.5%). Among these, *Staphylococci spp* exhibited a notable resistance rate of (26.29%), while *K. pneumonia* and *E. coli* showed even higher resistance, at (38.11% and 34.13%), respectively, against a range of antibiotics (16).
In a similar investigation in Sabha, Libya, in 2007, it was reported that 178 urine samples (68.5%) were contaminated with *E. coli* along with other microorganisms. Among the *E. coli* strains, 170 (65.4%) demonstrated susceptibility to nitrofurantoin, 157 (60.4%) to gentamicin, and 116 (44.6%) to cephalexin. However, only 6 (2.3%) strains were sensitive to erythromycin and 14 (5.4%) to tetracycline [17]. A study conducted at the Sabha Medical Center in 2018 examined strains of *Staphylococcus aureus* (MRSA) and found that out of 43 isolates of *Staphylococcus species*, (16%) were identified as MRSA. These strains exhibited resistance to erythromycin and clindamycin. The researchers concluded that vancomycin is the only effective antibiotic for treating infections caused by multidrug-resistant MRSA [18]. Similarly, research by Wareg *et al.* in 2014 indicated that resistance to vancomycin was absent in in-patient MRSA strains, although 5% of outpatient MRSA strains showed resistance through disc-diffusion assays [19]. This implies that vancomycin remains a viable treatment option for MRSA infections.
Another study from Libya reported that out of 128 MRSA isolates, (24.2%) were resistant to clindamycin, and (63.2%) to erythromycin. The authors noted that clindamycin could still be an effective treatment for MRSA infections in Libyan hospitals. In summary, common bacterial infections such as *Staphylococcus spp., E. coli, Klebsiella spp., Streptococcus spp., and Pseudomonas spp.* have been isolated from various sources and exhibit resistance to many antibiotics, posing significant challenges in Libya. These findings are supported by a review analysis of infection prevalence and resistance within different hospital departments in the region [20]. Conduct research in Ecuador to analyze the epidemiology of antibiotic resistance in bacteria collected from both inpatient and outpatient samples. The findings indicated a significant level of antimicrobial resistance, reaching as high as (80%) for certain medications, with hospitalized patients exhibiting a higher resistance rate compared to outpatients. The predominant bacteria identified in this study were *S. aureus* and *E. coli*. These results establish a crucial reference point for understanding antimicrobial resistance and enhance the surveillance system, aiding in the development of empirical therapy guidelines tailored to local epidemiology [21]. Similar studies and guidelines are essential for Libya.

Conclusion

This study assessed factors contributing to antimicrobial resistance (AMR) in Gharyan City, Libya, with a focus on antibiotic misuse and bacterial resistance patterns. Results revealed widespread misuse of antibiotics, including poor adherence to treatment duration and timing. Infections were more prevalent among females (77%) than males (23%), and occurred more frequently in younger patients compared to the elderly. The most common bacterial isolates were *Staphylococcus* spp. (32%) and E. coli (27%), followed by *Klebsiella* spp. (24%), *Streptococcus* spp. (14%), and *Pseudomonas* spp. (3%). Resistance was highest in *Pseudomonas* spp. (100%), E. coli (83%), *Staphylococcus* spp. (76%), *Klebsiella* spp. (69%), and *Streptococcus* spp. (67%). Antibiotics showing the greatest resistance included Azithromycin (67%), Doxycycline (49%), Trimethoprim, Amikacin, and Tetracycline (38%), while the lowest resistance was observed with Streptomycin (6%), Nalidixic acid (3%), and Meropenem (2%).

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***Received**: 10-11-2025 - **Accepted**: 09-01-2026 - **Published**: 15-01-2026*

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Received*: 10-11-2025 -* ***Accepted****: 09-01-2026 -* ***Published****: 15-01-2026*