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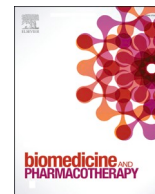
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Macrolide resistance outcomes after the Covid-19 pandemic: A one health approach investigation

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ABSTRACT

During the Covid-19 pandemic period, the indiscriminate use of macrolide-class antibiotics was frequent among the Brazilian population due to the lack of knowledge and information with a scientific basis. Thus, the class of drugs that includes azithromycin, clarithromycin, and erythromycin, which alter metabolic reactions in the body and act on the immune system, was widely used without medical prescription. Samples of bacterial strains from hospital environments were obtained during the most extensive spread of Covid-19 and studied in the present article, emphasizing the investigation for macrolide resistance genes (*erm* and *msr*) and bacteria of the genus *Staphylococcus* isolated from urinary tract infections. In addition, the physiological, genetic, immunological, and socio-epidemiological aspects were highlighted with a focus on the One Health approach and implications on the gut-brain axis in this integrative research, revealing that the inappropriate use of antibiotics directly affects entire communities, representing a significant concern for public and environmental health.

1. Introduction

Antimicrobial resistance (AMR) is the ability of a microorganism to resist the effects of medication previously and actively used to eliminate it, such as macrolide class chemicals (e.g., azithromycin, erythromycin, and clarithromycin). The indiscriminate use of these drugs interferes directly against resistant bacterial strains, considering the availability and the complex and slow process of creating new antibiotics [1]. Current antibiotics must maintain the lowest possible resistance profile to mitigate these effects, and the rationalization of their prescription has a predominant role in this objective [2].

Macrolides are bacteriostatic antibiotics; that is, they inhibit bacterial growth, triggering effective immune system mechanisms to

eliminate the pathogenic agents of the hypothetical infection acting by inhibiting the production of vital proteins [3]. In addition, macrolides will bind reversibly to a region of the larger subunit of the ribosomes (sites A and P) of the susceptible microorganisms, inhibiting the translocation of the chain of peptides, performed by a ribosomal protein called peptidyl transferase [4]. In this sense, the indiscriminate use of macrolides induces several mutations through biochemical and genetic mechanisms involved in bacterial resistance, such as mutations (alteration of the drug binding site), acquisition of resistance genes (mediated by plasmids and transposons), or efflux pumps that expel the drug from the bacterial cell [5,6]. In addition, the production of biofilms and the possibility of adaptation and tolerance by such pathogenic bacteria is a significant concern among clinicians and healthcare managers [7,8].

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Table 1

Classification of bacterial samples and resistance profile according to patient database.

Sex	Age (Years)	Identification of the sample	Microrganism	Sensitive standard dose	Sensitive increased dose	Resistance
Male	82	13668	<i>Staphylococcus warneri</i>	Gentamicin, linezolid, trimethoprim/sulfamethoxazole, and rifampicin	-	Penicillin, erythromycin, cefoxitin, levofloxacin, clindamycin, Erythromycin
Female	26	1242	<i>Staphylococcus xylosum</i>	Cefoxitin, gentamicin, clindamycin, linezolid, rifampicin, trimethoprim/sulfa, vancomycin, nitrofurantoin	Levofloxacin	Erythromycin
Female	37	14152	<i>Staphylococcus saprophyticus</i>	Cefoxitin, gentamicin, clindamycin, linezolid, rifampicin, trimethoprim/sulfa, vancomycin	Levofloxacin	Erythromycin
Female	44	18209	<i>Staphylococcus aureus</i>	Cefoxitin, gentamicin, linezolid, rifampicin, trimethoprim/sulfa	Levofloxacin	Clindamycin, erythromycin, penicillin
Female	77	20034	<i>Staphylococcus aureus</i>	Clindamycin, erythromycin, gentamicin, linezolid, rifampicin	Levofloxacin	cefoxitin, penicillin
Female	89	14645	<i>Staphylococcus epidermidis</i>	Cefoxitin, gentamicin, clindamycin, linezolid, rifampicin	Levofloxacin	Erythromycin, trimethoprim/sulfa
Female	90	20209	<i>Staphylococcus aureus</i>	Cefoxitin, gentamicin, clindamycin, linezolid, rifampicin, trimethoprim/sulfa	-	Erythromycin

The hindrance of bacterial resistance can be defined as a significant threat to public health, and it is paramount that actions against the exacerbated use of macrolides, considering the absence of dissemination of information about AMR in society, as they directly impact the future of humanity [9]. Then, considering the Covid-19 pandemic era, bacterial strains of uroculture origin, which exhibit resistance factors, are of great importance for understanding the environmental behavior of these pathogens during their infections, which are already common in communities [10].

Several factors contribute to Brazil's high resistance to macrolides and other antibiotics. In addition to the lack of strict regulation, the low public awareness and the dissemination of misinformation about the need for and effectiveness of antibiotics exacerbate the problem [11]. Furthermore, and in correlation with the One Health perspective, the Covid-19 pandemic has further compounded the misuse of antibiotics, as well as biocides, which have been linked to deadly co-infections caused by pan-resistant microorganisms belonging to the *Staphylococcus* genus (e.g., *S. aureus*, *S. saprophyticus*, and *S. epidermidis*) commonly found in human tissues causing skin infections, pneumonia, endocarditis, and osteomyelitis [12].

Many patients have received antibiotics as part of the treatment for symptoms associated with Covid-19, even when a bacterial infection was absent. This practice, regarding the macrolide resistance issue in communities, corroborates an even more worrying situation and urges immediate and coordinated measures to avoid an irreversible adverse scenario [13]. In addition, the Gut-brain axis is evidenced in this context in which the misuse of macrolides may interfere with natural microbiota, inhibiting part of the beneficial bacterial strains, causing immunological disorders, and releasing pro-inflammatory factors [14].

Hence, a phenotypic and molecular investigation of macrolide resistance factors in *Staphylococcus*, as well as their immunological implications, becomes necessary in this integrative research as a way of monitoring the pharmaco-ecological effects arising from reckless and neglected measures during the Covid-19 pandemic from the environment to the health of animals, such as the treatment of bovine, avian, porcine, and human bacterial infections [10,15]. The present study raises concerns about the mismanagement of antibiotic use in clinical and environmental situations and the One Health approach. It also focuses on its consequences on the gut-brain axis and immunological aspects by performing phenotypical and molecular assays on a significant number of bacterial strains.

2. Materials and methods

The present study has a descriptive-discursive character, emphasizing primarily the phenotypical and molecular resistance aspects

present in bacterial strains from clinical samples of patients who exhibited urinary tract infections in the pandemic scenario. Additionally, the widespread and uncontrolled abuse of macrolides and immunological and social information in the context were discussed. Thus, objective searches were performed in the following databases: PUBMED, SCIELO, and Google Scholar. In the search for the articles, the terms "Antimicrobial resistance", "Pandemic", "Urinary infections", and "Immunology of infections" were used without exclusion criteria. Still, the most recent articles were given greater attention due to their higher applicability.

Moreover, the clinical samples were obtained at the Hospital Unimed of Lorena municipality in São Paulo, Brazil. Initially, 38 bacterial samples (among 34 patients) with possible resistance profiles were revealed; however, a significant focus was given to urinary tract samples due to their greater antimicrobial resistance and the horizontal transfer of genetic mutations mediated by the environmental coexistence of the urinary tract pathogenic microbiota, in which, seven samples were submitted to molecular analysis (all strains information can be found in supplementary files section I). These samples were cultivated and identified, and their viability was proven within the Pontifical Catholic University, Poços de Caldas campus laboratories, Minas Gerais.

The sample size of the urinary tract bacterial strains was statistically supported by this study's highly reliable and valid results. The deliberate adoption of a high precision (99,9 %), low level of significance (0.001), and a one-sided test aimed to ensure a highly accurate estimative minimizing the probability of type I error and focusing specifically on the purposes of the research. This approach was carefully chosen to mitigate the sample size limitation while aiming to reach statistically substantial conclusions for the present study [16] (all mass spectrometry type MALDI-TOF / TOF test specifications can be found in supplementary files section II).

Bacterial samples were collected and tested for sensitivity profile using diffusion disc antibiogram (between September and December 2021), within the pandemic perspective, and subsequently, adequately frozen and preserved for subsequent studies. In addition, the samples originating from uroculture were genetically and phenotypically tested using the Polymerase Chain Reaction (PCR) method. Also, their use for scientific research was duly authorized via the declaration and term of the science of the Hospital Unimed of Lorena-SP (all PCR tests details can be found in supplementary files).

It is worth noting that the samples of urinary tract infections are MRSA strains (*S. aureus* resistant to methicillin) and were also tested against the following antibiotics: cefoxitin, gentamicin, clindamycin, linezolid, rifampicin, trimethoprim/sulfa, vancomycin, nitrofurantoin, levofloxacin, and penicillin, and if the profile of resistance to cefoxitin was present, they were resistant to all penicillins and penicillin-based

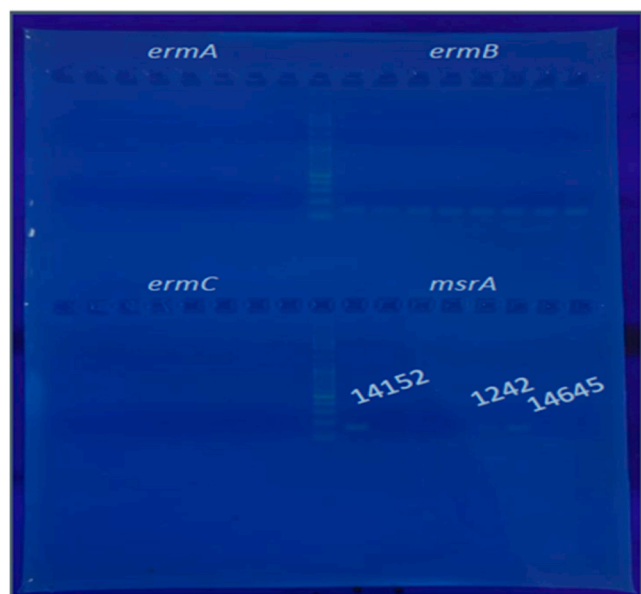


Fig. 1. Electrophoresis gel for PCR of *erm* and *msr* genes.

antibiotics. Finally, vancomycin sensitivity tests were performed using the concentration gradient tape method. The bacterial strains were cultured and averaged >100,000 CFU/mL count were obtained.

3. Results and discussion

In this integrative investigation, the results are separated into experimental analysis (Sections 3.1 and 3.2) and literature-based discussion (3.3–3.6) regarding the interconnections of macrolide resistance, immunological implications, gut-brain-axis and different aspects of health sciences, such as the One-health approach.

3.1. Phenotypical characterization of strains

The urine culture test with an antibiogram aimed to identify and count bacteria in samples, determining which antibiotics effectively control these bacteria. In the samples of urinary tract infections obtained from the Unimed Hospital of Lorena-SP, it was possible to identify the species: *Staphylococcus aureus*, *S. saprophyticus*, *S. hominis*, *S. haemolyticus*, *S. warneri*, *S. capitis*, and *S. epidermidis* (see supplementary material). The culture tests performed during the collection period and two years later proved the easy reproducibility of the species, which are fully viable and perform their primary metabolism. The counting averaged 100,000 CFU/mL.

The antibiogram tests revealed that the analyzed strains exhibited a resistance phenotype to penicillin, erythromycin, cefoxitin, levofloxacin, clindamycin, and trimethoprim/sulfamethox. In addition, they have increased dose sensitivity to levofloxacin. It is notorious that resistance to clindamycin is correlated to the resistance profile to all penicillins, as well as with Methicillin-Resistant *Staphylococcus aureus* (MRSA) strains (see Table 1).

According to Table 1, it is possible to observe that 85 % of the isolates were obtained from females, which corroborates the epidemiological data that show a higher occurrence of urinary tract infections in women [7]. Another relevant data is the prevalence of diseases in older adults (from 75 to 90 years of age), representing 57 % of the total individuals, while the other three samples were obtained from individuals below the middle-aged line (45 years). This data demonstrates older adults' greater immunological susceptibility, observed mainly by bacterial infections in the lower urinary genital tract.

Still, regarding the data obtained from the antibiograms, it can be

highlighted that 85 % of the strains are resistant to erythromycin, demonstrating the direct correlation with the presence of *erm* genes. However, the phenotypic resistance to the antimicrobial erythromycin was not observed in one sample, resulting in the absence of such a group of genes. Thus, the hypothesis of horizontal transfer of genetic elements is justified since such resistance possibly occurred by selective pressure accelerated by the abuse of macrolides during the pandemic.

3.2. Molecular analyses

The molecular analyses to investigate macrolide resistance consisted of detecting *erm* and *msr* genes in *Staphylococcus sp.* samples from clinical isolates of uroculture. The PCR endpoint results demonstrate that the *ermA*, *ermB*, and *ermC* genes were not detected, indicating that such genes were searched with primers corresponding to those compatible with the structural genome. Hence, it was investigated whether the gene was incorporated into the bacterial chromosome resistance to macrolides (Fig. 1).

Since there is no evidence of such genes (only dimerization of the primers referring to *ermB*, as also observed in the negative control), it is hypothesized that this resistance profile observed in the phenotypic tests is characterized by transient mechanisms and mobile elements that neutralize the action of macrolides in *Staphylococcus* species. Such evidence is followed by the amplification of the *msrA* marker in samples "14152", "1242", and "14645", with a size similar to the expected 163 bp. Thus, it is possible to deduce that this resistance may be mediated by efflux pumps (*mef*, *msr*, and *vga* genes) or there was only horizontal transfer of plasmid *erm* genes, in which they were not incorporated into the bacterial structural genome [17–19]. Therefore, *Staphylococcus sp.* represents an additional concern to clinicians because they exhibit erythromycin resistance factors, among several others, which drastically reduce therapeutic options.

The higher prevalence of macrolide resistance in women is a growing concern in several clinical sectors, as the irresponsible administration of azithromycin in pregnant women can be considered a risk factor regarding urinary tract infection without effective therapeutic options. A double-blind study, in which pregnant women were included for administration of placebo or azithromycin, was performed with an observation of the presence of *msrA* and *ermC* genes in the resistome of oropharyngeal secretion samples of newborns [20]. Due to the easy access and low toxicity of macrolides, such drugs are widely prescribed and dispensed by clinicians who attend the Brazilian health system and pharmacies. This further compromises the panorama of difficulty in treating resistant bacterial strains.

In this sense, overprescribing antibiotics for respiratory infections, often of viral origin, is a significant problem in pediatrics and is believed to play an essential role in increasing antibiotic resistance among bacterial pathogens [21]. Considering rare exceptions (occurrence of the immune storm), azithromycin is not recommended for viral infections, even with the risk of secondary bacterial infections, precisely because of the risk of the appearance of resistant strains [22]. Thus, the lack of information about the epidemiological management observed in several cities that distributed drugs of this class, with the neglected purpose of preventive protection against the SARS-CoV-2, favored the appearance of resistant strains, as observed in the results of this study.

Another study evaluated a screening technique for ribosomal mutations in Gram-positive bacteria that was observed by high-performance denaturation liquid chromatography (DHPLC) mutations in ribosomal genes in streptococci and staphylococci strains (*rplD*, *rplV*, and *rRL* genes associated with macrolide resistance). Portions of these genes were amplified by PCR from the total DNA of 48 strains, demonstrating that 37 were resistant to macrolides and had one or several mutations in one or two of the target genes. Thus, the presence of multiple factors in the mediation of macrolide resistance makes the investigation of such mechanisms increasingly complex for researchers [23].

3.3. Genetic mechanisms and physio-pharmacological aspects of macrolides resistance

Erythromycin is a macrolide that has 14 atoms in its lactone ring. From it was derived clarithromycin, a 14-membered compound that exhibits methylation of the hydroxyl group at position 6, and azithromycin, which, by adding nitrogen to its ring, results in a 15-membered compound [24]. Thus, resistance to these antibiotics by bacteria of the genus *Staphylococcus* may occur through genetic and adaptive mechanisms. To date, around 40 *erm* genes have been identified. Pathogenic bacteria carry these determinants mainly by self-transferable plasmids and transposons. Among the classes found in pathogenic microorganisms, four stand out: *erm*(A), *erm*(B), *erm*(C), and *erm*(F). The *erm*(A) and *erm*(C) gene classes are commonly found in *Staphylococcus* [25].

Erm(A) genes are often present in methicillin-resistant strains and carried by Tn554-related transposons, whereas *erm*(C) genes are generally responsible for erythromycin resistance in methicillin-vulnerable strains and are held by plasmids. The resistance phenotypes verified by the inducible expression of both determinants are similar and are characterized by dissociated resistance to antibiotics due to differences in their induction capacity. Therefore, the strains resist 14- and 15-membered ring macrolides, which are inducers [26].

Moreover, it is also known that the only efflux proteins that grant acquired resistance to macrolides characterized in *Staphylococcus* species are the ABC transporters encoded by plasmid-borne *msr*(A) genes, and the transmembrane component of the *msr*A pump remains unknown [27]. As detected in tests performed among the samples in this study, the resistance determinant *msr*(A) was first found in *Staphylococcus epidermidis* and has since been detected in several *Staphylococcus* species, including *S. aureus*. Thus, ABC transporters require ATP for their functioning and usually consist of a channel formed by two anchoring domains in the membrane and two ATP binding domains found on the cytosolic surface of the membrane [28]. The efflux system appears to have a multicomponent origin, including the *msr*A and chromosomal genes, to form a fully functional efflux pump, meaning the resistance is inducible [29].

In comparison to erythromycin, other macrolides, such as clarithromycin and azithromycin, offer better physiological tolerability; however, clarithromycin is more similar to erythromycin in pharmacokinetic characteristics, such as half-life (dwell time in the body), tissue distribution, and other drug interactions. Also, erythromycin may cause gastrointestinal side effects, such as nausea, vomiting, and diarrhea, compared to azithromycin, which is generally well tolerated. Hence, azithromycin has a lower interaction with the hepatic enzyme system, which may reduce the risk of drug interactions compared to erythromycin [30].

Due to their differences in the spectrum of activity, pharmacokinetics, and side effects, azithromycin and erythromycin can treat different types of infections. Also, erythromycin is often used against upper respiratory tract bacterial infections. In contrast, azithromycin is widely used for lower respiratory tract infections such as pneumonia, bronchitis and genital infections such as chlamydia [31]. Ribosomal methylation is the most widespread form of resistance by Erm proteins (erythromycin ribosome methylase). In pathogenic bacteria, these proteins methylate a single adenine in the nascent 23S ribosomal RNA, part of the 50S ribosomal subunit [32]. The overlapping binding sites of macrolides in 23S ribosomal RNA explain the cross-resistance of these drugs [33].

From other perspectives, resistance in Gram-negative bacteria may occur through antibiotic efflux caused by chromosomally encoded pumps that contribute to intrinsic resistance to hydrophobic compounds such as macrolides [34]. These pumps are often part of the resistance/modulation/division family formed by proteins with 12 anchoring regions in their membrane. As stated previously, in Gram-positive organisms (such as *S. aureus*), macrolide resistance by

active efflux is caused by two classes of pumps: members of the ATP-binding cassette transporter superfamily (ABC) and the superfamily of principal facilitators (MFS) [35].

Finally, drug inactivation resistance occurs only to antibiotics with a related structure and is caused by enzymes that can modify or break down the antibiotics, resulting in their inactivation. It is also worth mentioning that, so far, this specific type of resistance has not demonstrated considerable clinical relevance. However, considering a hypothetical future, it can still be threatened by public health managers during global infection occurrences [36].

3.4. Immunological reaction against bacterial *Staphylococcus* infections

Staphylococcus infections can be defined by the positive expression of the coagulase enzyme, which is an inhabitant of superficial tissues and is harmful to immuno-depressed people and those with autoimmune diseases [37]. Also, the genus *Staphylococcus* is highlighted as an agent of infection in the hospital environment and its species. Still, it has been commonly described as a cause of diseases that are difficult to control in public health environments worldwide. Hence, numerous virulence factors, combined with resistance to multiple classes of antimicrobials, endeavor to prevent infections caused by this microorganism in challenging situations [38].

Additionally, innate immune mechanisms are crucial in detecting and initial response to *Staphylococcus* infections. Overall, the innate immune cells (e.g., macrophages and dendritic cells) recognize microbial components, such as peptidoglycan and lipoteichoic acids, through pattern recognition receptors, such as Toll-like receptors (TLRs) [39]. The activation of these receptors triggers the production of pro-inflammatory cytokines, such as interleukin-1 β (IL-1 β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF- α), which recruit other cells and amplify the immune response [40]. In addition, macrophages are activated to phagocytize the invading bacteria; however, *S. aureus* and *S. epidermidis* have developed strategies to escape from these events, including the production of immunoglobulin (Ig) binding proteins that block opsonization and complement binding, and the formation of biofilms, which confer protection against phagocytosis and antimicrobials [41].

Furthermore, *S. aureus* can be considered one of the genus's main pathogenic species and has various immune evasion mechanisms. The production of a wide range of toxins, such as alpha-hemolysin toxins, leukocidins, and superantigen toxins, contributes to the destruction of immune cells, causing tissue damage and exacerbated inflammation [42]. *S. aureus* can also avoid the immune response by secreting proteases that degrade immune factors such as antibodies and cytokines. In clinical aspects, *S. aureus* can form abscesses and persist intracellularly in immune cells, such as macrophages and neutrophils, preventing their elimination. This intracellular ability allows *S. aureus* to evade the immune system and establish chronic infections. In addition, it has an evasion mechanism called "molecular camouflage", in which it modifies its surface to prevent recognition by immune cells' pattern-recognition receptors. This event complicates immune cell activation and elaborates an adequate response [43].

S. epidermidis, on the other hand, is considered a commensal species, generally found on the skin and mucous membranes. However, in certain conditions, such as the presence of implanted medical devices, it can cause opportunistic infections. One of the main strategies for immunological evasion of *S. epidermidis* is the formation of biofilms. These biofilms, consisting of bacterial layers wrapped in a polysaccharide extracellular matrix, confer protection against immune and antimicrobial responses, making eradicating infection even more threatening [44].

In addition, *S. epidermidis* can produce enzymes, such as catalase and superoxide dismutase, which neutralize reactive oxygen species, thus preventing oxidative damage to immune cells. It can also interfere with the activation of the complement system, preventing the formation of

complexes of attack on the membrane and cytotoxic action. Therefore, recent studies indicate that macrolide misuse correlates with decreased immune markers, such as IL-5 expressed in a lower form in CD4+ lymphocytes of patients treated with azithromycin.

Accordingly, the metabolic markers of individuals treated with drugs of this class demonstrated a particular elevation, indicating that the adequate physiology of the human organism was unbalanced through changes caused by macrolides in the intestinal microbiota [45]. Thus, it can be inferred that macrolides are closely linked to the intestinal microbiome and can alter it, implicating physiological changes of great relevance. Given such immunological implications, the clinical-epidemiological picture may worsen with the neglected use of antimicrobials, especially of the macrolide class [46].

3.5. Gut-brain-axis

Based on recent literature findings, the concept of the gut-brain axis consists of bidirectional communication between both organs, in which essential immune, metabolic, neurological, and endocrine functions are developed and constantly regulated. To ensure protection against pathogens, unique bactericidal peptides can be produced by the intestinal epithelium as innate mechanisms for the first defense barrier, consequently avoiding abnormalities in this important axis [47]. In addition, cells of the innate immune system present in the gut-associated lymphoid tissue (GALTs) are responsible for the non-specific recognition of pathogens, the initiation of the immune response, and the consequent activation of the adaptive immune system.

The intestinal microbiota is colonized mainly by bacteria of the phylum Firmicutes and Bacteroidetes. It performs essential functions in the immune system, influencing the differentiation of T lymphocytes into different types of helper T cells or regulatory T cells. In addition, it can induce the production of secretory immunoglobulin A (SIgA) through modulation in its main immunological epitopes and shaping the systemic immune response through signaling molecules, assisting in blood cell production and stimulating responses against the infectious process [48].

In addition, the gut microbiota protects against colonization by invading pathogens by releasing metabolites and bacteriocins capable of inhibiting protein synthesis and bacteria-associated peptide glycan synthesis [49]. It is also worth mentioning that dysbiosis in gut microbiota can be associated with the development and prevalence of auto-immune and inflammatory diseases [50] and metabolic disorders [51]. In this sense, it is possible to infer that changes in the intestinal microbiota can be harmful, causing a negative impact not only on the immune system but increasing susceptibility to different diseases.

Dysbiosis can be categorized as the presence of abnormalities related to the gut microbiome, and its origin is closely linked to several factors, such as inflammation or enteric infection, which significantly decreases the ability of the gut microbiota to prevent colonization by pathogens. Other factors such as high-fat and low-fiber eating habits, intestinal colonization at the time of newborn delivery (especially by the mode of delivery), pregnancy, and physical injuries directly impact intestinal dysbiosis [52].

The indiscriminate or inappropriate use of antibiotics, especially in childhood, is strongly linked to changes in diversity, taxonomic composition, and decrease of beneficial bacteria such as *Bifidobacteria* and *Lactobacilli*, as well as contributing to the increase in bacterial populations commonly related to urinary tract infections, such as *E. coli* [53]. Moreover, the effects of antibiotic use are connected to a decrease in bacterial diversity since several bacteria susceptible to the drug are destroyed, replaced by resistant bacteria, and other factors, such as increased antimicrobial tolerance and metabolomic changes [54].

Regarding the information mentioned above, there is growing concern related to the indiscriminate use of antibiotics, considering it affects not only human health but also animal and environmental health since they are intrinsically interconnected. In this sense, it is essential to

mention that antimicrobial resistance occurs not only through human action but the overuse of antibiotics in animals contributes significantly to antimicrobial resistance in enteric human bacteria, such as *Escherichia coli* and *Salmonella*, which are also related to animal normal microbiota. In addition, strains of methicillin-resistant *Staphylococcus aureus* (MRSA), a common pathogen for humans, have already been found in different animal species, which act as transmitters of this bacterium [55].

Regarding environmental health aspects, it is notable that pathogens of significant clinical and epidemiological relevance are commonly found in soil and wastewater, as well as antibiotics, which are considered emerging micropollutants, and, in contact with such pathogens, they may develop resistant strains. One of the main classes of drugs found in sewage or wastewater is macrolides, which are used in both humans and animals and are excreted through the bile and feces of these patients. This class of antibiotics has diverse mechanisms by which antimicrobial resistance may occur, representing a substantial concern for public health [56].

3.6. One health approach and social aspects of macrolide abuse

It is notorious that in Brazil, the administration of the macrolide class of antibiotics, in most cases, is not performed correctly, from medical prescription to self-medication [57]. The consumption of macrolides in Brazil is correlated with social and economic factors, including a lack of access to essential health services, standardized medical prescriptions, the absence of specific information for their best use, and social stigmas involving antibiotics in general [58].

Notably, the access to medicines, including erythromycin and azithromycin, can be affected by social factors such as economic inequality. Individuals with socioeconomic vulnerabilities have difficulty acquiring these medications, which directly impacts their ability to treat infections [59]. In addition, the prescribing patterns of erythromycin and azithromycin may vary in different social contexts and health systems. Occasionally, these antibiotics may be prescribed improperly or excessively, contributing to bacterial resistance and other public health problems [60].

In this sense, specific factors of paramount importance contribute to the poor consumption practices of macrolides. Generally, azithromycin and erythromycin are antibiotics commonly prescribed and dispensed, impacting the patient's use for self-medication [61]. It is relevant to consider such social aspects to promote the appropriate use of erythromycin and azithromycin, ensuring the effectiveness of treatment and minimizing the risks associated with bacterial resistance. Awareness, health education, and equitable access to medicines are crucial to addressing these social issues related to antibiotic consumption and seeking a unique health approach [62].

Considering bacterial infections in the urinary tract and the use of macrolides by individuals, it can be observable that the excretory system releases a large amount of antibiotics retained in the urine [63]. In addition to the incorrect disposal of medicines, in which waste is carried to water collection areas, standard water treatment systems do not ensure the removal of substances present in antibiotics, requiring plasma filtration technology, advanced oxidation processes, and reverse osmosis technologies (which are expensive and inaccessible technologies) for communities and countries without robust financial resources) to remove these substances that accelerate the phenomenon of microbial resistance [64].

In this regard, the water cycle continues without the total withdrawal of antibiotics, supplying cities, plantations, animal husbandry, and breeding sites. Irrigation of crops that use water without complete treatment leaves remnants of antibiotics in vegetables, legumes, and fruits that humans consume [65]. In addition, animals that need to drink water for survival and reproduction acquire doses of antibiotics. Thus, common antibiotics cannot treat a bacterial infection due to resistance factors that the excretory system eliminates [66].

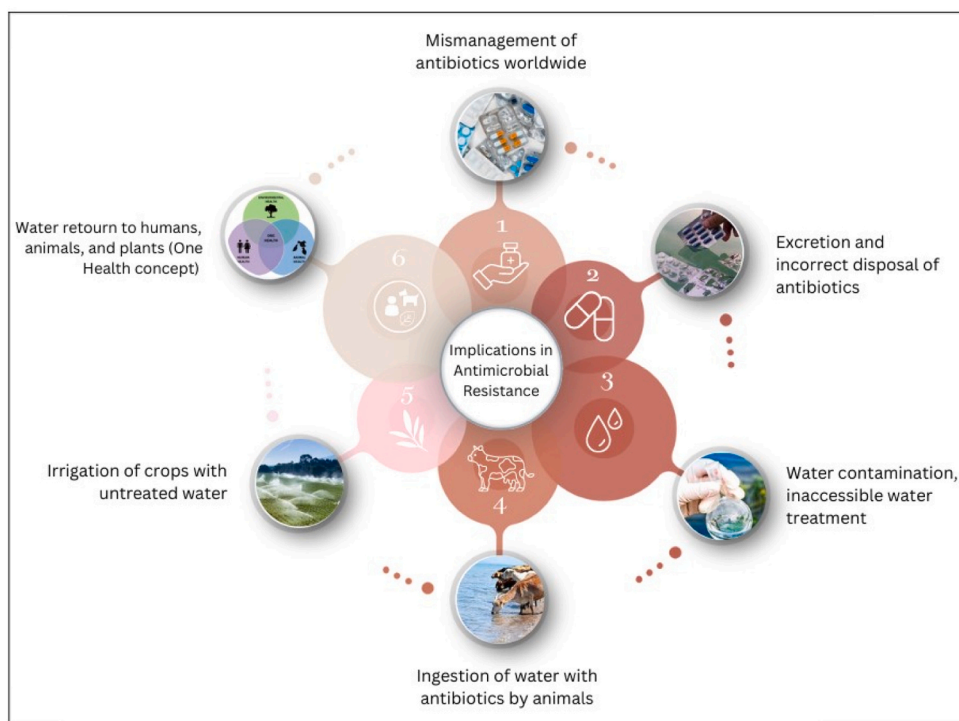


Fig. 2. Antibiotic cycle in the One Health context.

After this cycle, as shown in Fig. 2, bacteria remain increasingly resistant, causing bacterial infections that are untreatable or extremely difficult to treat. Hence, it is worth noting that the incorrect disposal of antibiotics can reach the marine portions, causing bacterial resistance in aquatic animals [67]. The One Health approach must be applied globally to prevent bacteria from continuing to mutate and cause resistance, as in urinary tract infections.

4. Conclusions

In summary, this study underlines the importance of assertive healthcare politics concerning all the aspects involving the mismanagement of antibiotics, including macrolides, during a global infectious disease crisis. It also enhances the immunological implications, both innate and adaptative reactions, in genito-urinary tract bacteriosis by selecting strains affected by horizontal gene transference mediated by plasmids. In addition, the gut-brain-axis microbiota association becomes evident with the experimental findings of antimicrobial resistance in this study, considering it plays an essential role in immune system modulation, aggravating the Covid-19 pandemic scenario in Brazilian hospitals. Finally, the biological cycling of macrolides is described in the One Health approach, which considers a broader extension of multidrug-resistant bacteria standoff along generations.

CRediT authorship contribution statement

Lidiane Godoy: Writing – review & editing, Methodology, Formal analysis. **Alex Sandro Lima:** Validation, Supervision, Resources, Project administration, Methodology, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Dalia Fouad:** Writing – review & editing, Resources, Funding acquisition. **Michel De Waard:** Writing – review & editing, Resources, Funding acquisition. **Tamara Santos:** Writing – review & editing, Methodology, Formal analysis. **Gaber Batiha:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition. **Jorge Pagnossa:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding

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Declaration of Competing Interest

The authors declares that there is no conflict of interest regarding the publication of this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at [doi:10.1016/j.biopha.2024.117437](https://doi.org/10.1016/j.biopha.2024.117437).

References

- [1] F. Prestinaci, P. Pezzotti, A. Pantosti, Antimicrobial resistance: a global multifaceted phenomenon, *Pathog. Glob. Health* 109 (7) (2015) 309–318.
- [2] R. Bland, S.R.B. Brown, J. Waite-Cusic, J. Kovacevic, Probing antimicrobial resistance and sanitizer tolerance themes and their implications for the food industry through the *Listeria monocytogenes* lens, *Compr. Rev. Food Sci. Food Saf.* 21 (2) (2022) 1777–1802.
- [3] P.H. Patel, M.F. Hashmi, *Macrolides*. StatPearls [Internet], StatPearls Publishing, 2023.
- [4] Omura, S., 2002. Macrolide antibiotics: chemistry, biology, and practice, second ed. Elsevier, Tokyo.

- [5] M.R. Schroeder, D.S. Stephens, Macrolide resistance in *Streptococcus pneumoniae*, *Front. Cell. Infect. Microbiol.* 6 (2016) 98.
- [6] Í.R. Garcia, F.A.O. Garcia, P.S. Pereira, H.D.M. Coutinho, A. Siyatadpanah, R. Norouzi, F.F.G. Rodrigues, Microbial resistance: the role of efflux pump superfamilies and their respective substrates, *Life Sci.* 295 (2022) 120391.
- [7] R.R. Muder, C. Brennen, J.D. Rihs, M.M. Wagener, A. Obman, A. Obman, V.L. Yu, Isolation of *Staphylococcus aureus* from the urinary tract: association of isolation with symptomatic urinary tract infection and subsequent staphylococcal bacteremia, *Clin. Infect. Dis.* 42 (1) (2006) 46–50.
- [8] J.P. Pagnossa, S.D.O. Rodrigues, G.F.D. Oliveira, M. Adnan, M.S. Aljaid, I.B. D. Assis, G.E.S. Batiha, COVID-19 in a pre-omicron era: a cross-sectional immunological and genomic evaluation, *Vaccines* 11 (2) (2023) 272, <https://doi.org/10.3390/vaccines11020272>.
- [9] S.O. Rodrigues, G.F. Oliveira, J.C. Franco, I.B. Assis, K. Banwo, J.P. Pagnossa, One health and a computational biology approach, *Res., Soc. Dev.* 11 (14) (2022) e02111437105, <https://doi.org/10.33448/rsd-v11i14.37105>.
- [10] Yadigaroglu, M.; Gorgun, S.; Yucel, M.; Guzel, M. The Effect of the COVID-19 Pandemic on Urine Culture Results and Resistance to Antibiotics in the Emergency Department. *Clinical Laboratory* 2022, 68(6). <https://10.7754/Clin.Lab.2021.211012>.
- [11] C.L. Ventola, The antibiotic resistance crisis: part 1: causes and threats, *Pharm. Ther.* 40 (4) (2015) 277.
- [12] H.J.A. Sulayyim, R. Ismail, A.A. Hamid, N.A. Ghafar, Antibiotic resistance during COVID-19: a systematic review, *Int J. Environ. Res Public Health* 19 (19) (2022) 11931, <https://doi.org/10.3390/ijerph191911931>.
- [13] S.S. Andrade, H.S. Sader, R.N. Jones, A.S. Pereira, A.C. Pignatari, A.C. Gales, Increased resistance to first-line agents among bacterial pathogens isolated from urinary tract infections in Latin America: time for local guidelines? *Mem. órias do Inst. Oswaldo Cruz* 101 (2006) 741–748.
- [14] S.O. Rodrigues, A.G. Pádua, C.M.G. Lima, Gut-brain axis as a key aspect of clinical conditions, *Int J. Fam. Commun. Med.* 6 (5) (2022) 285–289.
- [15] S.A. McEwen, P.J. Collignon, Antimicrobial resistance: a one health perspective, *Antimicrob. Resist. Bact. Livest. Companion Anim.* (2018) 521–547.
- [16] L. Flight, S.A. Julious, A practical guide to sample size calculations: installation of the app SampSize, *Pharm. Stat.* 21 (5) (2022) 1109–1110.
- [17] L. Jacobsen, A. Wilcks, K. Hammer, G. Huys, D. Gevers, S.R. Andersen, Horizontal transfer of tet (M) and erm (B) resistance plasmids from food strains of *Lactobacillus plantarum* to *Enterococcus faecalis* JH2-2 in the gastrointestinal tract of gnotobiotic rats, *FEMS Microbiol. Ecol.* 59 (1) (2007) 158–166.
- [18] S.J. Sorensen, M. Bailey, L.H. Hansen, N. Kroer, S. Wuerzt, Studying plasmid horizontal transfer in situ: a critical review, *Nat. Rev. Microbiol.* 3 (9) (2005) 700–710.
- [19] N.A. Lermniaux, A.D. Cameron, Horizontal transfer of antibiotic resistance genes in clinical environments, *Can. J. Microbiol.* 65 (1) (2019) 34–44.
- [20] A. Bojang, S.L. Baines, B. Camara, R. Guerillot, L. Donovan, R.S. Marqués, A. Roca, Impact of intrapartum oral azithromycin on the acquired macrolide resistance of infants' nasopharynx: a randomized controlled trial, *Clin. Infect. Dis.* 71 (12) (2020) 3222–3225.
- [21] C.L. Byington, H. Castillo, K. Gerber, J.A. Daly, L.A. Brimley, S. Adams, A.T. Pavia, The effect of rapid respiratory viral diagnostic testing on antibiotic use in a children's hospital, *Arch. Pediatr. Adolesc. Med.* 156 (12) (2002) 1230–1234.
- [22] G.E.S. Batiha, M.A. Zayed, A.A. Awad, H.M. Shaheen, S. Mustapha, O. Herrera-Calderson, J.P. Pagnossa, A.M. Algamal, M. Zahoor, A. Adhikari, I. Pandey, S. T. Elazab, K.R.R. Rengasamy, N. Cruz-Martins, H.F. Hetta, Management of SARS-CoV-2 infection: key focus in macrolides efficacy for COVID-19, *Front. Med.* 8 (2021) 642313, <https://doi.org/10.3389/fmed.2021.642313/full>.
- [23] A. Canu, A. Abbas, B. Malbruny, F. Sichel, R. Leclercq, Denaturing high-performance liquid chromatography detection of ribosomal mutations conferring macrolide resistance in gram-positive cocci, *Antimicrob. Agents Chemother.* 48 (1) (2004) 297–304.
- [24] T.L. Peeters, Erythromycin and other macrolides as prokinetic agents, *Gastroenterology* 105 (6) (1993) 1886–1899.
- [25] A.M. Ammar, A.M. Attia, M.I. Abd El-Hamid, I.M. El-Shorbagy, S.A. Abd El-Kader, Genetic basis of resistance waves among methicillin resistant *Staphylococcus aureus* isolates recovered from milk and meat products in Egypt, *Cell. Mol. Biol.* 62 (10) (2016) 7–15.
- [26] K. Kadlec, A.T. Fessler, T. Hauschild, S. Schwarz, Novel and uncommon antimicrobial resistance genes in livestock-associated methicillin-resistant *Staphylococcus aureus*, *Clin. Microbiol. Infect.* 18 (8) (2012) 745–755.
- [27] S. Hassanzadeh, M.R. Pourmand, R. Mashhadi, K. Ghazvini, Epidemiology of efflux pumps genes mediating resistance among *Staphylococcus aureus*; a systematic review, *Microb. Pathog.* 139 (2020) 103850, <https://doi.org/10.1016/j.micpath.2019.103850>.
- [28] E.D. Reynolds, J.H. Cove, Resistance to telithromycin is conferred by *msr (A)*, *msrC* and *msr (D)* in *Staphylococcus aureus*, *J. Antimicrob. Chemother.* 56 (6) (2005) 1179–1180.
- [29] F. Iannelli, F. Santoro, M. Santagati, J.D. Docquier, E. Lazzeri, G. Pastore, G. Pozzi, Type M resistance to macrolides is due to a two-gene efflux transport system of the ATP-binding cassette (ABC) superfamily, *Front. Microbiol.* 9 (2018) 1670.
- [30] G.W. Amsden, Erythromycin, clarithromycin, and azithromycin: are the differences real? *Clin. Ther.* 18 (1) (1996) 56–72.
- [31] C. Smith, O. Egunso, I. Choonara, S. Kotecha, E. Jacqz-Aigrain, H. Sammons, Use and safety of azithromycin in neonates: a systematic review, *BMJ Open* 5 (12) (2015) e008194, <https://doi.org/10.1136/bmjopen-2015-008194>.
- [32] R. Leclercq, P. Courvalin, Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification, *Antimicrob. Agents Chemother.* 35 (7) (1991) 1267–1272.
- [33] G. Maravic, Macrolide resistance based on the Erm-mediated rRNA methylation, *Curr. Drug Targets-Infect. Disord.* 4 (3) (2004) 193–202.
- [34] K. Poole, Efflux-mediated antimicrobial resistance, *J. Antimicrob. Chemother.* 56 (1) (2005) 20–51.
- [35] F. Van Bambeke, E. Balzi, P.M. Tulkens, Antibiotic efflux pumps, *Biochem. Pharmacol.* 60 (4) (2000) 457–470.
- [36] R. Leclercq, Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications, *Clin. Infect. Dis.* 34 (4) (2002) 482–492.
- [37] J.O.S. Nogueira, J.D. da Silva, J.G. de Pascoa Júnior, T.C.R. Pereira, C.C.A. Bezerra, A.C. Nogueira, *Staphylococcus aureus* resistente em unidade de terapia intensiva, e7312642006-e7312642006, *Res. Soc. Dev.* 12 (6) (2023), <https://doi.org/10.33448/rsd-v12i6.42006>.
- [38] W. Ziebuhr, *Staphylococcus aureus* and *Staphylococcus epidermidis*: emerging pathogens in nosocomial infections, *Contrib. Microbiol.* 8 (2001) 102–107.
- [39] S.L. Brandt, N.E. Putnam, J.E. Cassat, C.H. Serezani, Innate immunity to *Staphylococcus aureus*: evolving paradigms in soft tissue and invasive infections, *J. Immunol.* 200 (12) (2018) 3871–3880.
- [40] L. De Vor, S.H. Rooijackers, J.A. Van Strijp, *Staphylococci* evade the innate immune response by disarming neutrophils and forming biofilms, *FEBS Lett.* 594 (16) (2020) 2556–2569, <https://doi.org/10.1002/1873-3468.13767>.
- [41] C. Garzoni, W.L. Kelley, *Staphylococcus aureus*: new evidence for intracellular persistence, *Trends Microbiol.* 17 (2) (2009) 59–65.
- [42] Karauzum, H.; Datta, S.K. Adaptive Immunity Against *Staphylococcus aureus*. *Curr Top Microbiol Immunol* 2017, 409, 419–439. Doi: 10.1007/82_2016_1. PMID: 26919865; PMCID: PMC5534385.
- [43] N.W. De Jong, K.P. Van Kessel, J.A. Van Strijp, Immune evasion by *Staphylococcus aureus*, *Microbiol. Spectr.* 7 (2) (2019) 10–1128, <https://doi.org/10.1128/microbiolspec.gpp3-0061-2019>.
- [44] Le, K.Y.; Park, M.D.; Otto, M. Immune Evasion Mechanisms of *Staphylococcus epidermidis* Biofilm Infection. *Front Microbiol.* 2018, 9, 359. Doi: 10.3389/fmicb.2018.00359. PMID: 29541068; PMCID: PMC5835508.
- [45] J.M. Choo, A.M. Martin, S.L. Taylor, E. Sun, F.M. Moberg, T. Kanno, A. Richard, L. D. Burr, S. Lingman, M. Martin, D.J. Keating, A.J. Mason, G.B. Rogers, The impact of long-term macrolide exposure on the gut microbiome and its implications for metabolic control, *Microbiol Spectr.* 11 (4) (2023) e0083123, <https://doi.org/10.1128/spectrum.00831-23>.
- [46] A.E. Namvar, S. Bastarahang, N. Abbasi, G.S. Ghehi, S. Farhadbakhtarian, P. Arezi, M. Hosseini, S.Z. Baravati, Z. Jokar, S.G. Chermahin, Clinical characteristics of *Staphylococcus epidermidis*: a systematic review, *GMS Hyg. Infect. Control* 9 (3) (2014) Doc23, <https://doi.org/10.3205/dgkh000243>.
- [47] Q. Yuan, W.A. Walker, Innate immunity of the gut: mucosal defense in health and disease, *J. Pediatr. Gastroenterol. Nutr.* 38 (5) (2004) 463–473, <https://doi.org/10.1097/00005176-200405000-00001>. PMID: 15097431.
- [48] L. Wang, L. Zhu, S. Qin, Gut microbiota modulation on intestinal mucosal adaptive immunity, *J. Immunol. Res* 2019 (2019) 4735040, <https://doi.org/10.1155/2019/4735040>. PMID: 31687412; PMCID: PMC6794961.
- [49] M. Zhao, J. Chu, S. Feng, C. Guo, B. Xue, K. He, L. Li, Immunological mechanisms of inflammatory diseases caused by gut microbiota dysbiosis: a review, *Biomed. Pharmacother.* 164 (2023) 114985, <https://doi.org/10.1016/j.biopha.2023.114985>.
- [50] W.K. Mousa, F. Chehadeh, S. Husband, Microbial dysbiosis in the gut drives systemic autoimmune diseases, *Front Immunol.* 13 (2022) 906258, <https://doi.org/10.3389/fimmu.2022.906258>.
- [51] P. Bandopadhyay, D. Ganguly, Gut dysbiosis and metabolic diseases, *Prog. Mol. Biol. Transl. Sci.* 191 (1) (2022) 153–174, <https://doi.org/10.1016/bs.pmbts.2022.06.031>. Epub 2022 Jul 22. PMID: 36270676.
- [52] M. Levy, A. Kolodziejczyk, C. Thaiss, et al., Dysbiosis and the immune system, *Nat. Rev. Immunol.* 17 (2017) 219–232, <https://doi.org/10.1038/nri.2017.7>.
- [53] L. McDonnell, A. Gilkes, M. Ashworth, V. Rowland, T.H. Harries, D. Armstrong, P. White, Association between antibiotics and gut microbiome dysbiosis in children: systematic review and meta-analysis, *Gut Microbes* 13 (1) (2021) 1–18, <https://doi.org/10.1080/19490976.2020.1870402>. PMID: 33651651; PMCID: PMC7928022.
- [54] J. Ramirez, F. Guarner, L.B. Fernandez, A. Maruy, V.L. Sdepanian, H. Cohen, Antibiotics as major disruptors of gut microbiota, *Front. Cell. Infect. Microbiol* 10 (2020) 572912, <https://doi.org/10.3389/fcimb.2020.572912>.
- [55] B. Aslam, M. Khurshid, M.I. Arshad, S. Muzammil, M. Rasool, N. Yasmeen, T. Shah, T.H. Chaudhry, M.H. Rasool, A. Shahid, X. Xueshan, Z. Baloch, Antibiotic resistance: one health one world outlook, *Front. Cell. Infect. Microbiol* 11 (2021) 771510, <https://doi.org/10.3389/fcimb.2021.771510>.
- [56] C. Mutuku, Z. Gazdag, S. Melegh, Occurrence of antibiotics and bacterial resistance genes in wastewater: resistance mechanisms and antimicrobial resistance control approaches, *World J. Microbiol. Biotechnol.* 38 (9) (2022) 152, <https://doi.org/10.1007/s11274-022-03334-0>.
- [57] T.C. Marques, A.M.M. Reis, A.E.B.D.C. Silva, F.R.E. Gimenes, S.P. Opitz, T.C. A. Teixeira, S.H.D.B. Cassiani, Erros de administração de antimicrobianos identificados em estudo multicêntrico brasileiro, *Rev. Bras. De. Ciências Farm. éuticas* 44 (2008) 305–314.
- [58] B.S. Kliemann, A.S. Levin, M.L. Moura, I. Boszczowski, J.J. Lewis, Socioeconomic determinants of antibiotic consumption in the state of São Paulo, Brazil: the effect of restricting over-the-counter sales, *PLoS One* 11 (12) (2016) e0167885.

- [59] M. Filippini, G. Masiero, K. Moschetti, Socioeconomic determinants of regional differences in outpatient antibiotic consumption: evidence from Switzerland, *Health Policy* 78 (1) (2006) 77–92.
- [60] J.N.D.P. Pereira, M.A. Rabelo, J.L.D.C. Lima, A.M.B. Neto, A.C.D.S. Lopes, M.A. V. Maciel, Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of *Staphylococcus* spp. of a university hospital in Recife, Pernambuco, Brazil, *Braz. J. Infect. Dis.* 20 (2016) 276–281.
- [61] G. Nepal, S. Bhatta, Self-medication with antibiotics in WHO Southeast Asian Region: a systematic review, *Cureus* 10 (4) (2018).
- [62] R.E.C. d'Oliveira, R.R. Barros, C.R.V. Mendonça, L.M. Teixeira, A.C.D. Castro, Antimicrobial susceptibility and survey of macrolide resistance mechanisms among *Streptococcus pyogenes* isolated in Rio de Janeiro, Brazil, *Microb. Drug Resist.* 9 (1) (2003) 87–91.
- [63] S. Anjum, S. Singh, L. Benedicte, P. Roger, M. Panigrahi, B. Gupta, Biomodification strategies for the development of antimicrobial urinary catheters: overview and advances, *Glob. Chall.* 2 (1) (2018) 1700068, <https://doi.org/10.1002/gch2.201700068>.
- [64] C. Adams, Y. Wang, K. Loftin, M. Meyer, Removal of antibiotics from surface and distilled water in conventional water treatment processes, *J. Environ. Eng.* 128 (3) (2002) 253–260.
- [65] R. You, A. Margenat, C.S. Lanzas, N. Cañameras, N. Carazo, L. Navarro-Martín, V. Matamoros, J.M. Bayona, S. Díez, Dose effect of Zn and Cu in sludge-amended soils on vegetable uptake of trace elements, antibiotics, and antibiotic resistance genes: human health implications, *Environ. Res.* 191 (2020) 109879, <https://doi.org/10.1016/j.envres.2020.109879>.
- [66] P.H. Serrano, Responsible use of antibiotics in aquaculture, *Food Agric. Organ. U. Nations, Rome v.* 469 (2005).
- [67] G.G.N. Thushari, J.D.M. Senevirathna, Plastic pollution in the marine environment, *Heliyon* 6 (8) (2020) e04709, <https://doi.org/10.1016/j.heliyon.2020.e04709>.