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Gram-Positive Bacterial Infections and Antibiotics Resistance in Jordan: Current Status and Future Perspective

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Abstract

Background: Antibiotic resistance is expanding worldwide at alarming rates. Middle East countries including Jordan have high prevalence of antibiotic resistance.

Aims: The main aims of this review are to summarize the situation with Gram-positive bacterial infections and antibiotic resistance in Jordan, identify areas where further investigation is required, and suggest strategies to combat antibiotic resistance.

Methods: A systematic literature search was conducted by two independent researchers using general and specific combinations of MeSH search terms using Embase, PubMed, Web of Science, and Google Scholar databases.

Results: Staphylococci and Streptococci were commonly isolated from environmental, animal, and human samples, while Staphylococci, Enterococci, and Listeria were commonly isolated from food. Staphylococci, Streptococci, and Enterococci human colonization were documented at variable but high rates. Methicillin-resistant *S. aureus* (MRSA) and methicillin-resistance coagulase-negative Staphylococci (MR-CoNS) infections were common with high rates of antibiotic resistance. *S. pneumoniae* showed increased resistance rates to most antimicrobials. Enterococci and *C. difficile* resistance rates were moderate, while group B Streptococci (GBS), viridans group streptococci (VGS), *C. perfringens* and *L. monocytogenes* antibiotic susceptibility patterns were not reported. All MRSA and vancomycin-resistant Enterococci (VRE) isolates were *mec-A* positive, while resistance genes among CoNS, *S. pneumoniae*, *S. pyogenes*, *S. agalactia*, *C. perfringens*, and *L. monocytogenes* were not investigated.

Conclusions: Gram-positive bacterial infections and antibiotic resistance rates were high in Jordan. Molecular epidemiology studies, a nationwide surveillance program, and action plans are urgently required to combat antibiotic resistance.

Key words: infection; antibiotic resistance; Jordan; MDR; gram-positive; bacteria.

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Introduction

The antibiotics era started with the discovery of penicillin by Fleming in the late 1920s. By the 1930s, most common infections were successfully treated with antimicrobials. Sulfonamide, a synthetic antimicrobial agent was first used in 1935. Trimethoprim, another synthetic agent, was developed in the early 1960s [1,2]. Penicillin resistance was first reported in late 1940s, and sulfonamide and Trimethoprim resistance were first identified in 1969. The exact mechanism, including genetic background, was not known at that time [1,2]. The origin of modern antibiotic resistance genes in pathogens is possibly the environmental bacteria. Recent work has revealed resistant bacteria in ancient permafrost, caves, and preserved human specimens. An ancient origin of resistance that precedes antibiotic use has been proposed [3].

Multi-drug resistant (MDR) bacterial infections are usually associated with high morbidity and mortality as conventional antibiotics are of little value. Some bacterial strains have developed resistance to every known antibiotic, including last resort antibiotics. Antibiotic resistance is increasing at an alarming rate, especially in the last decade [4]. This challenge is clearly represented by the recent reports of WHO stating that antibiotic resistance is the most serious public health issue of our time [5,6]. Antibiotic resistance has multiple effects on social, economic, political, ethical, public health and clinical issues [7-10].

Now, multiple Gram-positive organisms are known to be resistant to multiple antibiotics, including *Staphylococcus aureus*, *Clostridium difficile*, Vancomycin-resistant *Enterococci* (VRE), and *Streptococcus pneumonia* [4,7,10]. Antibiotic resistance patterns can be different based on geographic location, socio-economic conditions, health care systems, and political engagement [8,10,11]. This is related to the prevalence of different infecting bacterial agents, the genetic background of bacterial strains, environmental factors including animals and food, policies and protocols for antibiotic prescriptions and strategies applied to combat antibiotic resistance [8,10,11]. Some countries have been able to contain resistance, while in most countries, the prevalence of antibiotic-resistant bacteria is increasing [5,8]. Most of the action planes to tackle antibiotic resistance have originated in the USA and Europe, while some countries are still in the development stage of an action plan. Other countries have no effective measures or clear strategies to control antibiotic resistance [8,11].

Multiple studies conducted in the Mediterranean region have shown a high prevalence of antibiotic resistance, especially in the eastern and the southern regions [8,12,13]. The Mediterranean area is experiencing a surge in *S. aureus* and *S. pneumonia* antibiotic resistance [14-16]. The lack of organized antibiotic resistance surveillance programs, effective strategies to combat antibiotic resistance, the progressive increase in antibiotic resistance at national levels and uncontrolled antibiotics prescription have a global impact [10].

Multinational studies in southern and eastern Mediterranean countries showed the overall prevalence of penicillin non-susceptible *S. pneumoniae* was 26% with the highest proportions being from Algeria and Lebanon [12,15]. Iran is the only Mediterranean country with documented tolerance and/or resistance to vancomycin in *S. pneumoniae* [16]. The Middle East is considered endemic for methicillin-resistant *S. aureus* (MRSA) carriage and infections, with sequence type 80-MRSA-IV becoming more common [17]. MRSA rates varied

from 10% in Lebanon to 65% in Jordan, while vancomycin non-susceptibility was detected in 0.9% of *E. faecalis* isolates from Turkey and in 3.8% of *E. faecium* isolates from Cyprus [12,14].

Few literature reviews, systematic reviews, and meta-analyses have been published on Gram-positive antibiotic resistance overall, or resistance patterns for specific organisms in Syria [18], Turkey [19], Saudi Arabia [20-23]and Kuwait [24]. No reviews have been reported in Jordan so far. International, regional, and local studies have indicated a higher prevalence of antibiotic resistance in Jordan. The highest proportions of MRSA among Mediterranean countries were previously reported by Jordan, Egypt and Cyprus, where more than 50% of the invasive isolates were methicillin-resistant [14]. Similarly, the rates of antibiotic resistance in *S. pneumonia* isolates from Jordan is among the highest in the world[25].

The main aim of this review is to systematically summarize the current state of Gram-positive infections and antibiotic resistance patterns and molecular types in Jordan, identify gaps and areas where further investigation is required, and finally suggest strategies and plans to combat antibiotic resistance.

Methods

A comprehensive literature search was conducted using general and specific combinations of MeSH search terms. The search engines used without limits include Embase, PubMed/Medline, Web of Science, and Google Scholar. General combination terms including "infection/s", "bacteria", "micro/organism", "antibiotic", "antibiotic resistance", "microbial drug resistance", "MDR", "antimicrobial resistance", "AMR", "Gram positive", and "Jordan" were used. Specific combinations terms including each organism's name, for example "Staphylococci", "Staphylococcus", "*Staphylococcus aureus*", "*S. aureus*", "methicillin-resistant *Staphylococcus aureus*", "MRSA", and "Jordan" were also used. Each article title and abstract were reviewed by two independent researchers for relevance to review aims. Inclusion criteria included: 1) Original studies or systematic reviews about Gram-positive bacterial infection and/or colonization of the environment, food and water, animals and birds, or humans in Jordan, 2) Antimicrobial resistance of Gram-positive bacterial colonization and/or infection of humans in Jordan, 3) Prevalence, antibiogram, phenotype, and genotype of antibiotic resistance for each Gram-positive organism in Jordan. Relevant full text articles were extracted and included in the study. Articles related only to infection and/or colonization were described briefly, while articles related to antibiotics resistance, especially in clinical studies, was approached in more depth.

Staphylococcus

Staphylococci is a Gram-positive cocci that mediates large number of human infections including skin, soft tissue and wound infections, deep seated infections, gastrointestinal infections, urinary tract infections (UTIs), and others[26]. Staphylococci is mainly classified into coagulase-positive Staphylococci (CoPS) which includes *S. aureus* and the coagulase-negative Staphylococci (CoNS) which includes *S. epidermidis*, *S. haemolyticus*, and other species[27,28]. Carriers of *S. aureus* mainly through anterior nares are common and play essential role in transmission of infections [28]. Resistance of Staphylococci has been documented to all antibiotics commonly used, including more recent findings of resistance to glycopeptides, linezolid, and daptomycin, and is a major concern[26]. The WHO considers the development of new drugs

for MRSA and Vancomycin-resistant *Staphylococcus aureus* (VRSA) as a "high priority"[29]. Methicillin resistance in *S. aureus* is mediated by Staphylococcal cassette chromosomal mec (SCCmec) which carries the *mecA* gene and includes multiple Staphylococcal protein A (Spa) subtypes[30], while vancomycin resistance is mediated by *vanA*, *vanB*, and other *van* genes[28,31].

S. aureus was isolated from the environment in Jordan, including in the air at six Hospitals[32], the hands and mobile phones of university students[33,34], the hands of food handlers[35], waterpipe drain hoses[36], the storage cases of contact lenses[34], and the organic and inorganic archaeological objects in Jordanian museums[37]. Similarly, *S. aureus* was isolated from different foods in Jordan, including traditional dairy products and milk [38-40], different types of meat[41-45], imported fish[46], cheddar cheese [47], and table eggs[48]. Furthermore, *S. aureus* was isolated from animals in Jordan, including dogs[49], camels [40,42,50], slaughtered goats[51], dairy cows with mastitis[52-54], bovine[55,56], dairy cattle's[57,58], Awassi sheep[59-62], and heifers[63]. It was also isolated from pneumonic sheep lung[64], camels' cutaneous abscesses [65], and the lung abscesses [66]and arthritic joints of calves[67], and it was associated with neonatal mortality in lambs and kids of sheep and goats, respectively (Table 1)[68].

S. aureus was isolated from multiple clinical samples in Jordan including blood, wounds, abscesses, skin, ears, urine, stool, the upper respiratory tract (URT), eyes, and others[69-78]. It was the main cause of health care-associated bloodstream infections[79], wound infection[80-82], surgical site infections[83,84], intensive care infections[85], otitis media[86], ocular bacterial infections[87], lower respiratory tract infections[88], bacteremia[89-91], neonatal septicemia[92,93]and septic arthritis[94]. *S. aureus* was among the most common isolates in infected burns[95,96], bacteremia in cancer patients with febrile neutropenia[97], cancer patient infections in general[98], biliary tract infections[99], and nosocomial infections[100]. It was a rare isolate in UTIs[101-107], otitis externa[108-110], and ventilator associated pneumonia (VAP)[111]. Few cases of *S. aureus* infections were reported in Jordan in the following illnesses: neonatal meningitis[112], recurrent meningitis in children[113], nosocomial neonatal septic arthritis[114], endocarditis[115], bilateral primary psoas abscesses[116], orbital abscess with acute ethmoiditis [117], primary pyomyositis[118,119], intramedullary spinal cord abscess[120], persistent bacteraemia[121], sinusitis with orbital cellulitis[122], tufted hair folliculitis[123], combat-related traumatic chronic osteomyelitis[124], and autoimmune hepatitis and transverse myelitis presented with persistent bacteremia (Table 1)[125].

Multiple studies have investigated the prevalence of nasal colonization with MRSA among different populations and age groups in Jordan. Adults' colonization rates were 4.3%[31], 7.5% [30], 19%[70], 22.7%[81], and 24.6%[77]. Children's colonization rates were 7.1% [126] and 7.2%[31], and infants' rate was 7.9%[127]. MRSA colonization rates among health care workers and medical students were 2.4%[128], 5.8%[129], 8.7%[130] and 10.1%[31]. Colonization among adult hospitalized patients was 6.4%[28]. Furthermore, MRSA was detected in 5.3% of stool samples from infants[127], 40% of nasal and stool samples in general[130], and 5.7% on the skin of healthy volunteers[30]. All MRSA nasal isolates from Jordan were *mecA*-positive [28,31,70,128], mostly were SCCmec type IV[30,31,126,127], and Spa types t223, t9519, and t044 were the most common[30,31,126,130]. Panton-Valentine leukocidin (PVL) was prevalent in nasal isolates at 0%[126], 0.9%[30], 5.4%[31], 15%[130], and 28%[127]. A large

proportion of nasal isolates was resistant to ampicillin and benzylpenicillin while all isolates were sensitive to vancomycin (Table 1)[28,30,31,70,127,128].

Isolates from clinical samples in Jordan have higher rates of MRSA, including 8.8%[69], 31.6%[76], 49.5%[72], 56%[14], 57%[70], 62%[71], 67.3% and of hospital-acquired (HA) infections[77], 68% [74] and 73.2% [78], and were all mediated by the *mecA* gene[31,70,71,77,78]. MRSA clinical isolates in Jordan possessed the SCCmec type III or IV[73,131], with Spa type t932 and t044 being the most common[73,74,131]. MRSA clinical isolates had 76.7% inducible resistance to macrolide-lincosamide-streptogramin B (iMLSB), 4.7% macrolide-steptogramin B-resistant phenotype (MSB), and 18.6% constitutive MLSB mediated by *ErmA, ermB* and *ermC* genes[77]. MRSA isolates from clinical samples were highly resistant to penicillin (94.6%)[76], erythromycin (61, 77, 78.2 and 100%)[70,73,76,77], clindamycin (79 to 100%)[70,74], chloramphenicol (98%), and levofloxacin (85%)[74], moderately resistant to streptomycin, kanamycin, and fusidic acid (36%)[73], while only 2.6% were mupirocin resistant mostly mediated by *mupA* gene[71]. While most studies reported no resistance to vancomycin[70,76,78,132], a single case of erythrodermic psoriasis developed persistent bacteraemia due to vancomycin-intermediate *Staphylococcus aureus* (VISA)[121], one case of bacteremia in cancer patients was intermediate to vancomycin[97], and 5 cases out of 139 (3.6%) from Zarqa governorate were VRSA [133]. Overall, 25% of *S. aureus* isolates were MDR and 67% were extensively drug-resistant (XDR)[74]. *S. aureus* resistance to linezolid, daptomycin, fifth generation cephalosporins, and other new antibiotics was not investigated in Jordan.

Clinical isolates exhibited a high prevalence of virulence genes, toxin genes, and adhesion genes[78,134]. Isolates contained at least 4 toxin genes, and 60% of them contained 12-13 genes[78]. Seventy-eight percent had the PVL gene, 84% carried the exfoliative toxin gene, 19 to 26% had the Toxic Shock Syndrome Toxin gene[73,81], and 23 to 39% had enterotoxin genes[135,136].

CoPS, including 88.57% *S. aureus*, 6.8% *S. intermedius*, and 4.57% *S. hyicus*, were isolated from humans (26% nose, 16% nail, and 73% clinical), and animals (6.25% nose, 17.1% meat, and 8% milk). Five isolates were resistant to vancomycin, including two isolates from humans and three isolates from animal meat. The MDR rate was higher in clinical samples and animal meat and milk [75].

CoNS are common commensals on the skin and mucous membranes and can cause a wide variety of opportunistic and nosocomial infections[137]. CoNS have been isolated from bovine samples and samples from sheep with mastitis[56-63], the hands and mobile phones of university students[33], air samples[138,139] and health care workers' uniforms in ICUs[140]. The most common CoNS species isolated from clinical samples in Jordan were *S. epidermidis* and *S. haemolyticus*[141], and these were isolated from neonates with septicaemia[92,93,142-144], nosocomial septic arthritis patients[114], cancer patients with bacteremia and febrile neutropenia[97,145], patients with bloodstream infections[132,141], patients with nosocomial infections[100], patients with UTIs[102,104,106,107,146-148], patients with otitis externa[110], children with bacterial infections following autologous hematopiotic stem cell transplantation[149], patients with surgical site infections following coronary artery bypass graft surgery[150], and patients with endocarditis[115].

Methicillin-resistant-CoNS (MR-CoNS) carriage was 54.2% (nose 48.5% and skin 12.3%) in a healthy Jordanian population with *S.*

epidermidis SCCmec type IVa predominating[30]. MR-CoNS isolates were susceptible to gentamicin (97.4%), tetracycline (90.8%), norfloxacin (84.9%), and clindamycin (79.6%), while 52.6% were resistant to erythromycin, and 7.2% had inducible clindamycin resistance[30]. Nasal colonization by CoNS among hospitalized patients was 14.8%, mostly by *S. haemolyticus* and *S. sciuri* with 73.3% of MR-CoNS harboring the *mecA* gene. Isolates were highly resistant to benzylpenicillin, erythromycin, fosfomycin, and imipenem, but they were all sensitive to vancomycin and were negative for the *vanA* and *vanB* genes[27]. Multi-drug resistant nasal CoNS were higher in non-cephalosporin plant workers compared to the cephalosporin plant workers (Table 1)[151].

Among CoNS clinical isolates, MR-CoNS rate was 31%[132], high resistance was reported for ampicillin, penicillin, ceftriaxone, cefazolin, gentamicin, cefepime, amoxicillin-clavulanic acid, and erythromycin[132,141,143], while very low or no resistance was reported for imipenem, rifampin, nitrofurantin, mupirocin, linezolid, teicoplanin, and vancomycin[71,132,141,143]. Antibiotic resistance genes, including *mecA* and *van* genes, have not been investigated in clinical infections with CoNS in Jordan.

Streptococcus

Streptococcus is a Gram-positive coccus belongs to the family Streptococcaceae and classified based on capsular carbohydrate structure and ability to induce hemolysis of blood agar. It causes many infections including pharyngitis, eye infections, meningitis, endocarditis, dental caries, and pneumonia. Antibiotic resistance in Streptococci has increased in recent years including resistance to penicillin, beta-lactam cephalosporins, macrolides, and fluoroquinolones[152].

Streptococcus spp was isolated from animals with mastitis in Jordan including bovine and ovine animals[55,56,153], camels[40,50], dairy cattle [57,58], dairy cows[52], Awassi sheep[59-61], and heifers[63]. Furthermore, it was isolated from pneumonic sheep lung[64] and camels' samples from cutaneous abscesses[65], lung diseases [66,154,155], nasal cavity[154], liver abscesses[154,155], and arthritic joints (Table 1)[67].

Streptococcus spp was also isolated from table eggs[48], hot springs [156], the dominant hand and mobile phones of University students[33], university student's mobile phones, contact lens storage cases, and conjunctiva of contact lenses users[34], and nurses and health care workers uniforms in intensive care units (ICU)[140] in Jordan.

Streptococci was a common cause of otitis media in children[86], bacterial infections in children after transplantation of autologus hematopiotic stem cell[149], infections in adults with cancer[98], and endocarditis patients[115]. It was a rare causative agent of bacteremia in children[89,91], UTI [102,105,147], proptosis in young Jordanians[157], and poststreptococcal glomerulonephritis and rheumatic fever[158]. Group D Streptococcus (*S. bovis*) was isolated from surgical site infections[83], group B, C, and G were isolated from pharyngitis[159], *S. oralis* was isolated from health care associated blood stream infections[79], *S. fecalis* was isolated from neonatal septicemia[93], and *S. milleri* was isolated from UTI [146]. β-hemolytic Streptococcus was isolated from otitis externa[108], and UTI[101], while α-hemolytic Streptococcus was isolated from neonatal sepsis[143] and community acquired UTI (Table 1)[160].

S. pneumoniae is an asymptomatic colonizer of the nasopharynx of healthy individuals[161]. Colonization was variable ranging from 14% to 55% according to location and age of the studied population in Jordan; including 55.1%

of children aged 1 to 50 months in Amman[162], 14.0% in Amman and 41.2% in Madaba in children 2 to 144 months[163], 29.0% in Irbid and 37.4% in Madaba in children 2–4 years[164], 33.8 to 37.8% in children under 5 years of age in north Jordan[161,165], and 19.5% in children from kindergartens and pediatric clinics in Amman[166]. High resistance rates of *S. pneumoniae* nasopharyngeal isolates were documented in Jordanian studies for penicillin (52% to 91.8%), erythromycin (46.7% to 61.5%), clarithromycin (54.7% to 81.3%), oxacillin (64.4% and 90.6%), trimethoprim-sulfamethoxazole (54.7% to 100%), and tetracycline (32.3% and 53.8%). Low resistance rates were noted for clindamycin (19.5% and 33.8%), cefotaxime (0% and 29.2%), ceftriaxone (2.3%), chloramphenicole (0% and 6.2%), and ciprofloxacin (6.2%). Full susceptibility was noted for vancomycin (100%), levofloxacin, amoxicillin (100%), and telithromycin (100%)[161,162,164,166]. High rates of macrolide resistance, clindamycin resistance, and macrolide licosamide-streptogramin B (MLS_B) resistance were also documented in nasopharyngeal isolates [161,162,164,166]. Macrolide resistance was mediated by *erm(B)*, *mef(A)*, and *mef(E)* genes[161,162,166], and was associated with 19F, 6B, and 23F serotypes[161,162,166]. Multidrug-resistance rates of nasopharyngeal isolates were 34.4% to 43.8% in Amman, 54.4% to 68.9% in Madaba, and 78.9% in Irbid (Table 1) [162-164,166].

S. pneumoniae was the causative agent for a wide variety of life-threatening infections in Jordan including pneumonia, meningitis, and bacteremia[25,89,90,113,167-170]. *S. pneumoniae* was most frequently isolated from eye specimens (21.0%), bloodstream infection samples (16.7%), and sputum samples (14.6%) with invasive isolates accounting for 23.6% of all isolates[25]. In addition, *S. pneumoniae* was a leading cause of otitis media[171], sinusitis, bronchitis[162], ocular bacterial infections[87], and lower respiratory tract infections[88,172]. It was occasionally involved in health care associated blood stream infections[79] and neonatal septicemia[93]. Susceptibility test results from1298 invasive isolates of *S. pneumoniae* from blood and spinal fluid cultures showed dual non-susceptibility to penicillin and erythromycin in excess of 5% in laboratories from Algeria, Tunisia, Lebanon, Jordan, and Turkey[15]. Clinical isolates of *S. pneumoniae* observed from 1982 to 1999 in Jordan University Hospital (JUH) showed an increase in resistance rates to all antimicrobials tested, except for vancomycin[173]. Similarly, *S. pneumoniae* isolated from 22.4% outpatient children with otitis media in north Jordan showed a significant decline in susceptibility to tobramycin[171]. MDR was 8.6% among pneumococcal isolates from JUH with an increased prevalence of non-susceptibility to erythromycin, clindamycin and levofloxacin over the study period of 19 years[25]. The susceptibility of Streptococcus organisms was more than 50% to amoxicillin-clavulanic acid, ampicillin, cefaclor, cefixime, cephalothin, cotrimoxazole, ciprofloxacin, cefotaxime, gentamicin, piperacillin, erythromycin, lincomycin, tobramycin, and vancomycin[25,171,173], while low susceptibility rates were observed to penicillin (43%-62%)[173]. Resistance to vancomycin reported in one study was unexpected[171]because other studies reported 100% susceptibility to vancomycin[25,173]. Antibiotic resistance genes relevant to *S. pneumoniae* were not investigated in Jordan (Table 1).

Group A streptococcus (GAS) (*S. pyogenes*) was a common colonizer of the nasopharynx[174], the most common cause of URT infections[172]and pharyngitis[159,174], and a rare isolate from surgical site infections[83] and UTI[107]. It was highly resistant to penicillin

(87.9%), and ampicillin (65.7%), moderately resistant to erythromycin (40%), and rarely resistant to cefuroxime (7.4%), vancomycin (9.1%), gentamycin (11.4%), cotrimoxazole (13.5%), and ciprofloxacin (14.5%)[172].

Group B streptococcus (GBS) (*S. agalactia*) can normally colonize the vagina and cause infections in both the mother and the fetus including chorioamnionitis, neonatal septicemia, meningitis and osteomyelitis [92,94,112,144]. Vaginal-rectal swabs from women who presented for labor and delivery at Al-Bashir Hospital showed that 19.5% were positive for GBS with serotype group III being the most common[175], while about 30.4% had positive vaginal and rectal colonization during late pregnancy[176]. GBS was associated with UTI[102,106,177]and surgical site infections[83,150]. 33% of multi-drug resistant serious bacterial infections in the first 90 days of life in King Abdullah University Hospital (KAUH) were caused by Gram-positive bacteria with GBS being the most common[178]. There are reports emerging in the MENA region of increasing erythromycin and clindamycin resistance in GBS (Table 1)[175].

Viridans group streptococci (VGS) commonly colonize the oral cavity, cause dental caries, and are an opportunistic pathogen leading to subacute infective endocarditis and bacteremia[179-181]. Eighty-one out of 146 oral swaps from patients attending periodontal clinic at JUH had VGS including *S. mitis* (27%), *S. mutans* (13.6%) and *S. salivarius* (12.3%). Twenty-three of the VGS isolates were erythromycin-resistant, 44% harbored erythromycin resistance genes, and 32% were positive for endocarditis antigen (efaA)[180]. VGS was associated with sinusitis with orbital cellulitis[122], neonatal sepsis[92,142], otitis externa infections[110], and febrile neutropenia (in a single case in a pediatric cancer patient)[145], and infective endocarditis in two cases[115]. Vancomycin-resistant *Streptococcus thoraltensis* was isolated from the nasal cavity of a healthy young adult university student in Jordan[182] and was isolated from an abdominal wall abscess in a young female. The sample from the young female showed resistance to ampicillin, oxacillin and gentamycin (Table 1)[183].

Enterococcus

Enterococci are a common commensal organism of human intestine and has increasingly become a major nosocomial pathogen[184]. The emergence of vancomycin-resistant Enterococci (VRE) is of major concern due to limited treatment options and the possibility of transmission to other species including Staphylococci[100]. Multiple studies have documented the wide spread of Enterococci in Jordan. *Entrococcus faecalis* was isolated form hands, conjunctiva, and mobile phones of Jordanian students[33,34], airborne in healthcare setting[138], oral rinse specimens from dental diseases[185], and were detected in traditional drinks[186], raw cow milk[38], commercial chickpea dip[187], and turmus (ready-to-eat lupin seeds)[188] consumed in Jordan. Enterococcus was isolated from dairy cows and Awassi sheep with mastitis (Table 1)[52,57,58,61].

Enterococcus colonized the intestinal tract of 72% of non-hospitalized infants and 28% of hospitalized infants and was resistant to ampicillin (6.5%, 30%), chloramphenicol (20%, 25.8%), and leveofloxacin (9.7%, 30%) for *E. faecalis* and *E. faecium,* respectively, while susceptible to vancomycin, teicoplanin, and linezolid[189]. Enterococci were isolated form different clinical samples[12], bacteremia in children[89], bloodstream infections[79,132], chemotherapy-induced febrile neutropenia with positive blood cultures[97], bacterial infections in the first 90 days of life[178], skin and soft tissue

infections from ICU patients[85], wound infections[82], bile infections[99], and nosocomial infections[100]. It was rarely isolated from UTIs[101,102,104,106,107,146-148,160,177,190-193], dental diseases,[185], sinusitis and orbital cellulitis[122], combat-related traumatic chronic osteomyelitis[124], and autologous hematopoietic stem cell transplantation in children[149]. *E. faecalis* was the most frequent isolate followed by *E. faecium*[100,189].

Antibiotic susceptibility pattern of Enterococci in Jordan showed 0% resistance to chloramphenicol, amoxicillin-clavulanic, and teicoplanin, 0-4% to vancomycin, 1% to nitrofurantoin, 7.6% to penicillin-G, 0-15% to ampicillin, 17.7% to leveofloxacin, 0-28.6% to ciprofloxacin, 37.5% to erythromycin, 52.17% to streptomycin, 59.2-100 % to gentamicin, 70.1-77.17% to tetracycline, 78.5% to imipenem, 100% to cefazolin, trimethoprim/sulfamethoxazole, clindamycin and oxacillin[12,97,100,102,185,193]. Molecular studies showed that all VRE strains were harboring *vanA* gene[100], while all *E. faecalis* isolates from patients with dental diseases had collagen binding protein (*ace*) and endocarditis antigen (*efaA*) genes[185]. Intestinal colonization with Enterococci among children, adults or patients, recent data regarding VRE rates among colonization or infection samples, and occurrence of *vanB* and other *van* genes were not investigated in Jordan.

Clostridium

Clostridium difficile is an anaerobic, Gram-positive, spore-forming toxigenic bacterium, which causes infectious colitis[194]. This bacterium might also be carried asymptomatically in the gut, potentially leading to 'silent' transmission[195]. Major virulence factors are toxin A and toxin B carried through *tcdA* and *tcdB* genes, respectively, and binary toxin carried through *cdtA* and *cdtB* genes and detected predominately in more virulent strains like ribotype 027 (RT027)[194,195]. Resistance against antimicrobials used for *C. difficile* therapy such as metronidazole, vancomycin, fidaxomicin, fluoroquinolones and macrolides have been reported at variable rates worldwide[196].

The prevalence of toxin-positive *C. difficile* in stool samples was 14.63/1000 in discharged patients, 12.65% of patients, and 5.0/1000 patient-hospital day [197], 9.7% to 13.7% in adult hospitalized patients' stool[198,199], and colonized the gut of 12.9% of Jordanian paediatric patients[200]. *C. difficile* isolates had moderate resistance to fluoroquinolones, low resistance to macrolides, and no or very low resistance to metronidazole and vancomycin. *GyrA* and /or *gyrB* was detected in about 40% of isolates [199,200]. *C. difficile* isolates had 54 to 73% *tcdA* and/or *tcdB* toxin genes, one isolate had the binary toxin gene, while no ribotyping studies were reported in Jordan (Table 1)[199,200].

Clostridium perfringens isolates were reported from broiler chicken flocks and sheep flocks[201,202], lambs and kids in sheep and goat farms[68], slaughterhouse goat carcasses and liver abscesses[51], and liver abscesses of Awassi sheep in Jordan[203]. *C. perfringens* intestinal colonization rate among Jordanian infants was 27.2%, predominantly of genotype A, with resistance rates of 20% to metronidazole and erythromycin, 16.7% to levofloxacin, and 6.7% to vancomycin[204]. Enterotoxigenic *C. perfringens* was isolated from 2% of acute diarrhea patients reporting to hospitals and health centers in northern Jordan[205] and burn wound infections[96].

Clostridial infections were reported in goats with pneumonia[51] and increased neonatal mortality in small ruminants[206]. *C. colinum* was

isolated from broiler flocks with digestive disease[207]. Also, *C. tetani* causing tetanus following a burn injury was reported in an 18-month-old girl [208]. Also, both *C. tetani* and *C. septicum* were isolated from burn wound infections[96]. No studies on *Clostridium botulinum* were reported in Jordan.

Listeria

Listeria species are small rod-shaped Gram-positive bacteria that include 17 recognized species of them. *Listeria monocytogenes* is the most common. Listeria causes food poisoning, meningoencephalitis, abortion, and septicemia. Although antibiotic resistance in Listeria species remains low, multidrug-resistant strains have been documented in food, animal, and human isolates[209].

L. monocytogenes was isolated from different food sources in Jordan including 1.5%, 2.7%, 5.3%, 17.1%, 18.2%, and 21.9% of Ready-to-Eat (RTE), raw, and processed chicken and beef[210-214], 31.5% in fish[215], 7.5%, 11.5% and 45% in raw and bulk tank milk[216-218], 4% to 35% in cheese[47,217,219], 40% in dry yoghurt[217], and one isolated from egg shell surface[48]. Other Listeria species including *L. welshimeri*, *Listeria ivanovi*, *L. grayi*, and *L. innocua* were also isolated from RTE meat and chicken products, raw and processed meat products, milk and dairy products[210-214,216,217,219]. *L. monocytogenes* was isolated from 7 archaeological objects in a Jordanian museum[37]. *L. monocytogenes* was prevalent in 7.1% of anorectal mucosal swabs and fecal samples from imported beef cattle in Jordan[220,221]. The farm-level prevalence of *L. monocytogenes* among dairy cattle farms in Jordan was 27.9% (Table 1)[218,221].

Multidrug resistance was exhibited by 96.9% of *L. monocytogenes* isolates form dairy cattle farms[218], 57% of isolates from RTE meat products[212], 73.1% isolates from fish[215], and 100% of isolates from beef cattle[220]. More than 50% of *L. monocytogenes* isolates from food or animals were resistant to ampicillin, clindamycin, penicillin, erythromycin, quinupristin-dalfopristin, streptomycin, teicoplanin, linezolid, vancomycin, kanamycin, fosfomycin, oxacillin, fusidic acid, neomycin, and tetracycline[215,218,219]. While more than 90 % of the *L. monocytogenes* isolates from beef cattle and fish resisted fosfomycin, oxacillin, ampicillin, penicillin and erythromycin, and more than 75% resisted vancomycin[215,220]. In contrary, *L. monocytogenes* isolates from raw and processed meat products had low resistance (≤10%) to ampicillin, gentamicin, vancomycin, tetracycline, kanamycin, and erythromycin[213]. Despite the high occurrence of multidrug resistant-*L. monocytogenes* in food and animal studies, the prevalence and antibiotic susceptibility of *L. monocytogenes* from clinical isolates were not reported in Jordan.

Causes of increased antibiotics resistance in Jordan and future perspective

Antibiotic misuse, overuse, and improper use, purchasing antibiotics without medical prescriptions, inadequate community knowledge and malpractice[222,223-227], improper use of antibiotics in the food industry[16], and improper use with animals[222,228] all lead to high rates of antibiotics resistance in Jordan. Jordan had the highest reported rate of self-medication with antibiotics among the Euro-Mediterranean region[229].

A high percentage of MDR bacterial colonization exists within hospitals and the community[33,128,222]. There is a lack of proper phenotypic and molecular diagnostic testing according to guidelines, especially for colistin and vancomycin, where disc diffusion testing is still used[222,230]. There is a lack of effective surveillance

systems[16,17,230]. Poor implementation of infection control guidelines[231], treatment guidelines[232], regulation to restrict antibiotic access[233], and antibiotic stewardship programme[17,234] are all factors in these problems.

Data on antibiotic resistance in the Middle East region are generally scarce with few studies performing genotyping analysis. According to WHO, none of the eastern Mediterranean region countries had national plans or progress reports concerning antimicrobial resistance for the last 5 years, and only 38% of these countries performed surveillance of bacterial resistance[16]. In the Mediterranean area, limited data are available on current overall epidemiology of MDR bacteria in livestock, companion, and domestic animals[235]. Nationwide studies on antimicrobial resistance are very limited.

Future efforts should focus on establishing national surveillance; plans, and research studies on antibiotic resistance; improving diagnostic capacity for antibiotic resistance; implementing infection control guidelines, treatment guidelines, antibiotic prescription regulations, and antibiotic stewardship programmes; and continuous education of health care providers and community[227].

Conclusions

Gram-positive isolates were reported from the environment, food, animals, birds, and humans in Jordan. Staphylococci and Streptococci are common causes of human colonization and infections in Jordan, yet most studies are from a single center on limited numbers. Phenotypic antibiotic susceptibility studies of Gram-positive bacteria showed increased resistance rates to most antimicrobials with limited data available for GBS, VGS, *C. perfringens* and *L. monocytogenes*. Furthermore, resistance genes among CoNS, *S. pneumoniae*, *S. pyogenes*, *S. agalactia*, *C. perfringens*, *L. monocytogenes*, and others were not investigated. Resistance to vancomycin among Gram-positive bacteria in Jordan is limited. Molecular epidemiology studies, a nationwide surveillance program, and the development of an action plan are necessary to fight antibiotic resistance.

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Conflict of interest statement

The authors declare no conflicts of interest.

Table 1: Summary of environmental, food, animal/birds, human colonization and infections, and antibiotic resistance studies on Gram-positive bacteria in Jordan. CoNS: Coagulase-negative staphylococci, GAS: group A Streptococcus, GBS: group B Streptococcus, VGS: viridans group streptococci.

	Environment	**Food**	**Animal/ bird**	**Human**		**Antibiotic Resistance**	
				Colonization	Infection	Phenotype	Genotype
S. aureus	Airborne of Hospitals[32] Mobile phones[33 34] Storage cases of contact lenses [34] Organic and inorganic Archaeological objects in Jordanian museum[37] Waterpipe device hoses[36]	Traditional dairy products and milk[39-40] Different types of meat [41-45] Imported fish [46] Cheddar cheese[47] Table eggs[48]	Dogs[49] Camel's[40,50,65-67] Cows[52 53,54] Bovine [55,56] Cattles [57,58] Sheep [59-62,64,68] Goats [51,68] Heifers [63]	**MRSA nose** Adults: 4.3%[31] (7.5%[30], 19%[70], 22.7%[81], 24.6% [77]. Children's: 7.1%[126], 7.2%[31] Infants: 7.9%[127] Healthcare workers: 10.1%[31], 5.8% [69,129] Medical students 2.4%[128] Adult hospitalized patients 6.4%[27]. **MRSA stool** Infants: 5.3%[127] **MRSA skin** healthy volunteers: 5.7%[30] **Others** Hands of food handlers[35] Hands and mobile phones of university students33,34	Mixed clinical samples[69-78] Health care-associated bloodstream infections[79] (Wound infections[80-82] Surgical site infections [83,84] Intensive care infections[85] Otitis media [86] Ocular bacterial infections [87] Lower respiratory tract infections[88] Bacteremia[89-91] Neonatal septicaemia and septic arthritis [92-94]. Infected burn [95,96]. Bacteremia and other infections in cancer patient [97,98]. Bile infections [99]. Nosocomial infections [100]. UTIs [101-107] Ventilator associated pneumonia (VAP) [111]. Otitis externa [108-110] Case studies or few cases [112-125].	**MRSA** 8.8%[69] ,31.6%[76], 49.5% [72], 56%[14], 57%[70], 62%[71], 67.3%[77] , 68% [74], 73.2% [78] **iMLSB** 76.7%, MSB 4.7%, 18.6% cMLSB [77]. **High resistance:** penicillin, erythromycin, clindamycin chloramphenicol, and levofloxacin [70,73,74,77] **Moderate resistance:** streptomycin, kanamycin, and fusidic acid [73], **Low/no resistance:** mupirocin and vancomycin [70,71,77,78,97,121,132,133]	***mecA* gene**[27,31,70,71,77,78 ,128] ***ermA, ermB* and *ermC* genes[77]** ***mupA* gene** [71].
CoNS	Airborn[138,139] Mobile phones of university students [33] Health care workers' uniforms in ICUs[140]	NA	Bovine, heifers, cattle and sheep mastitis[56-63]	**MR-CoNS nose** Healthy: 48.5%[30] Hospitalized patients: 73.3%[27] **MR-CoNS skin** Healthy 12.3%[30]	Neonatal septicaemia [92,93,142-144] Nosocomial septic arthritis[114] Bacteremia in cancer patient with febrile neutropenia[97,145] Blood stream [132,141] Nosocomial infections [100] UTIs[102,104,106,107,146-148] Otitis externa [110] Bacterial infections in children following transplantation of autologus hematopiotic stem cell[149] Surgical site infection[150] Endocarditis[115].	**MR-CoNS** 31%[132] **Highly resistance:** ampicillin, penicillin, ceftriaxone, cefazolin, gentamicin, ciprofloxacin,cefepime, amoxicillin-clavulanic acid, and erythromycin [132,141,143] **Low/no resistance:** imipenem, rifampin, nitrofurantin, mupirocin, linozolid, teicoplanin, and vancomycin[132,141,143]	NA

	Environment	Food	Animal/ bird	Human		Antibiotic Resistance	
				Colonization	Infection	Phenotype	Genotype
S. pneumoniae	Dominant hand and mobile phone[33].	NA	NA	Nasopharyngeal Colonization 55.1% [162], 14.0-41.2%[163], 29.0% and 37.4%[164],33.8[161], 37.8%[165], 19.5% [166]	Mixed clinical samples [25] Pneumonia, meningitis and bacteremia [25,89,90,113,167-170] Otitis media [171] Sinusitis, bronchitis [162] Ocular bacterial infections[87] Lower respiratory tract infections [88,172] Health care associated blood stream infections[79] Neonatal septicemia[93]	>50% susceptibility to Amoxicillin-clavulanic acid, ampicillin, cefaclor, cefixime, cephalothin, cotrimoxazol, ciprofloxacin, cefotaxime, gentamicin, piperacillin, erythromycin, lincomycin, and tobramycin[25,171,173] Vancomycin (susceptibility about 100%)[25,171,173] Penicillin susceptibility (43%-62%)[173] Dual non-susceptibility to penicillin and erythromycin > 5% [15]	NA
GAS	Student's university mobile phones, contact lenses storage cases, and conjunctiva of contact lenses users[34]	NA	NA	Nasopharynx colonization[174]	Upper respiratory tract infections[172] Pharyngitis[159,174] Surgical site infections [83] UTI [107]	**Highly resistant to:** penicillin (87.9%), and ampicillin (65.7%)[172] **Moderately resistant to:** erythromycin (40%) [172] **Rarely resistant to:** cefuroxime (7.4%), vancomycin (9.1%), gentamycin (11.4%), cotimoxazole (13.5%), and ciprofloxacin (14.5%) [172]	NA
GBS	NA	NA	Awassi sheep [59,61] Bovine and ovine[153] Dairy cattle [57,58] Dairy cows[52] Camels[40]	Vaginal colonization[94,175,176]	Chorioamnionitis, neonatal septicemia, meningitis and osteomyelitis [92,94,112,144] UTI [102,106,177] Surgical site infections[83,150] Serious bacterial infections in the first 90 days of life[178]	NA	NA
VGS	Nurses and health care workers uniform in intensive care unite [140]	NA	Camels[154 155]	Oral cavity colonization[179-181]	Subacute infective endocarditis and bacteremia[115,181] Sinusitis with orbital cellulitis [122] Neonatal sepsis [92,142] Otitis externa infections [110] Febrile neutropenia in pediatric cancer patients [145]	NA	NA

	Environment	**Food**	**Animal/ bird**	**Human**		**Antibiotic Resistance**	
				Colonization	Infection	Phenotype	Genotype
Enterococcus	Mobile phones of Jordanian students [33,34] Airborne in healthcare setting [138]	Traditional drinks [186] Raw cow milk [38] Commercial chickpea Dip [187] Turmus (ready-to-eat lupin seeds) [188]	Dairy cows and Awassi sheep with mastitis[52,57,58,61]	Hands and conjunctiva of Jordanian students[33,34] Oral rinse specimens from dental diseases[185] Intestinal colonization [189]	Mixed clinical samples[12] Bacteremia [79,89,97,132] Bacterial infections in the first 90 days of life[178] Skin and soft tissue infections [85] Wound infection [82] Bile infections [99] Nosocomial infections [100] UTIs [101,102,104,106,107,146-148,160,177,190-193] Dental diseases [185] Sinusitis and orbital cellulitis[122] Combat-related traumatic chronic osteomyelitis [115] Autologous hematopoietic stem cell transplantation in children [149]	**High resistance:** streptomycin, tetracyclin, cefazolin, trimethoprim/sulfamethoxazole [100,102,193] **Moderate resistance:** penicilin-G, gentamicin [100,102,193] **Low/no resistance:** ampicillin, chloramphenicol, leveofloxacin, ampicillin amoxicillin-clavulanic, ciprofloxacin, gentamicin, nitrofurantoin, tetracycline, vancomycin, teicoplanin [12,97,100,185]	***vanA* gene[100]**
C. difficile	NA	NA	NA	Colonized the gut of paediatric patients [200]	Hospitalized patient with diarrhea[197-199]	**Moderate resistance:** ciprofloxacin levofloxacin[199] **Low/no resistance:** metronidazole vancomycin [199]	***gyrA and gyrB genes*[199]**
C. perfringens	NA	NA	Broiler chicken and sheep[201,202] Lambs and kids in sheep and goat farms [68] Slaughterhouse goats with carcasses and liver abscesses[51] Liver abscesses of Awassi sheep [203]	Intestinal colonization in infants [204]	Acute diarrhea [205] Burn wound infection[96]	NA	NA
L. monocytogenes	Archaeological objects in Jordanian museum [37]	Ready-to-Eat, raw, and processed chicken and beef[210-214] Fish [215] Raw and bulk tank milk[216-218] Cheese[47,217,219] Dry yoghurt [217] Egg shell surface[48]	Beef cattle[220,221] Dairy cattle [218,221]	NA	NA	NA	NA

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الالتهابات البكتيرية موجبة صبغة جرام ومقاومتها للمضادات الحيوية في الأردن

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الملخص

خلفية البحث: مقاومة المضادات الحيوية تنتشر في كل أنحاء العالم بوتيرة متسارعة؛ إذ إنَّ دول الشرق الأوسط –بما فيها الأردن– تعاني من معدل انتشار عالٍ لمقاومة المضادات الحيوية.

الأهداف: الأهداف الرئيسة من هذه المراجعة العلمية، هي تلخيص الوضع الحالي للالتهابات البكتيري موجبة صبغة جرام ومقاومتها للمضادات الحيوية في الأردن، تحديد نقاط الضعف التي تحتاج إلى مزيد من الدراسة، واقتراح استراتيجيات للتغلب على مقاومة المضادات الحيوي في على المستوى المحلي.

طريقة البحث: مراجعة الأبحاث العلمية بطريقة منهجية بواسطة باحثين مختلفين، وباستخدام مصطلحات عامة وخاصة في محركات البحث امباس، بب ميد، ويب أوف سينس، وجوجل سكولر.

النتائج: بكتيريا المكورات العنقودية والعقدية منتشرة في البيئة والحيوانات والإنسان، بينما البكتيريا العنقودية والمكورات المعدية، وبكتيريا الليستيريا منتشرة في الأطعمة؛ حيث إنّ المكورات العنقودية والعقدية والمعدية منتشرة لدى الإنسان بدرجات مختلفة ولكنها عالية، أمّا المكورات العنقودية الذهبية المقاومة للميثيسيلين، والمكورات العنقودية السلبية المُخثِّرة المقاومة الميثيسيلين فهي منشره بكثرة في الأردن، بينما البكتيريا العقدية الرئوية لديها زيادة في مقاومة أغلب المضادات الحيوية، في حين أنّ بكتيريا المكورات المعوية والكوليستريديم لديها مقاومة متوسطة للمضادات الحيوية، ولا يوجد دراسات لمدى مقاومة المضادات الحيوية لبكتيريا العقدية باء، والعقدية فيريدانس وبيرفيرينجس وليستيريا في الأردن، وجميع المكورات العنقودية الذهبية المقاومة للميثيسيلين والمكورات المعوية المقاومه للفانكوميسين تحتوي على جين مقاومة المضادات الحيوية(ميك إي) بينما لا يوجد دراسات عن جينات مقاومة المضادات الحيوية في الأنواع الأخرى من البكتيريا موجبة الصبغة في الأردن.

الاستنتاج: الالتهابات البكتيرية موجبة صبغة جرام والمقاومة للمضادات الحيوية منتشرة بكثره في الأردن، وهناك ضعف في الدراسات البيئية الجينية على المستوى الوطني، واستحداث برنامج مراقبة وطني، ووضع استراتيجية وطنية لمقاومة المضادات الحيوي أصبح ضرورة ملحة في الأردن.

الكلمات الدالة: الالتهابات، مقاومة المضادات الحيوية، الأردن، البكتيريا موجبة صبغة جرام، البكتيريا متعددة المقاومة للمضادات الحيوية.