International Journal of Infectious Diseases 83 (2019) 77–82

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Exploring bacterial resistance in Northern Oman, a foundation for implementing evidence-based antimicrobial stewardship program

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ARTICLE INFO

Article history:
Received 18 January 2019
Received in revised form 31 March 2019
Accepted 1 April 2019
Corresponding Editor: Eskild Petersen, Aarhus, Denmark

Keywords:
Stewardship
Resistance
Susceptibility
Phenotypes
Oman

ABSTRACT

Background: Increasing rate of resistant infections is a challenge to healthcare negatively impacting therapeutic and financial outcomes. Targeted antimicrobial stewardship interventions are needed to counteract this global crisis. On large scale, we sought to identify the prevalence of resistant pathogens and their susceptibility pattern in Northern Oman.

Material and method: Retrospective analysis of all isolates processed by Suhar Hospital microbiology laboratory between Jan1st, 2016 and Dec31st, 2017. Organism identification, susceptibility and phenotyping were performed following CLSI standards and duplicate isolates were excluded. Pertinent microbiological data were collected and analyzed.

Results: Of 15,733 samples included, Gram-negative bacteria predominate by 67.76%, Gram-positive (29%) and *Candida* species (2.63%). Frequently isolated Gram-negative bacteria were *Escherichia coli* (32.39%), *Pseudomonas aeruginosa* (22.16%), *Klebsiellapneumoniae* (19.97%) and *Acinetobacter baumannii* (5.22%), there was virtually no resistance to colistin and tigecycline, while a growing resistance toward ciprofloxacin and meropenem was observed. Resistant *E. coli* and *K. pneumoniae* were isolated from bloodstream infection (12%). While Gram-positives were MSSA (27.23%), *Streptococcus agalactiae* (25.36%), MRSA (16.10%) and CoNS (12.1 %), they were almost universally susceptible to daptomycin and linezolid with low resistance (820%) to clindamycin. Approximately, 50% of Staphylococci (MRSA and CoNS) required vancomycin treatment.

Conclusion: Study findings should guide targeted stewardship interventions to optimize antibiotic prescriptions. Empirical treatment options should be revised, drug-bug match therapy instituted promptly and newer agents considered. Prescribing restriction of formulary antimicrobials that still retain their activity towards bugs – like colistin, linezolid and tigecycline- is a mandatory action. Review empiric use of ciprofloxacin and meropenem to counteract growing resistance.

Introduction

Bacterial resistance to antibiotics has grown to become a major challenge confronting healthcare providers (Hooper et al., 2012; Hawkey, 2008). Plentiful literature discusses the decreased bacterial susceptibility to almost all available antibiotics (Karam and Heffner, 2000). The 2013 report of United States Centers for Disease Control and prevention (CDC) declared that at least 2 million people in the USA acquire infections with resistant bacterial phenotypes and at least 23,000 people die yearly because of these infections (European Centre for Disease Prevention and Control, 2019). A study conducted on two groups of children with a community acquired urinary tract infection (UTI) 8 years apart showed a generalized decrease in bacterial susceptibility to common oral antibiotics; ampicillin, cephalexin

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https://doi.org/10.1016/j.ijid.2019.04.004

and nitrofurantoin 35% to 30%, 82% to 63% and 93% to 92% respectively (Prais et al., 2003).

The dramatic accrual of resistant bacterial pathogens concomitant with the dearth of novel antimicrobial agents incited Infectious Diseases Society of America (IDSA) to launch the 10 × '20 initiative that seeks a global commitment to create an antibiotic research and development (R&D) enterprise powerful enough to produce 10 new systemic antibiotics by the year 2020" (Infectious Diseases Society of America, 2010). Moreover, IDSA published a call for action to encourage the adaptation and adherence to antimicrobial stewardship programs in health care settings.

Research demonstrated the deleterious effect of infections caused by bacteria possessing certain resistance determinants. In 2016 a study conducted in USA revealed that the incidence of extended spectrum beta-lactamase (ESBL) producing *E. coli* infections increased from 5.28 to 10.5 (almost 100%) per 100,000 patient days during the 5-year study period (Thaden et al., 2016). ESBL production was a major predictor of mortality, prolonged length of stay, delay in initiation of appropriate antimicrobial therapy, and increased hospitalization costs (Boucher et al., 2009). In the USA in 2013, a 2-year study correlated increased prevalence of carbapenem-resistant Enterobacteriaceae (CRE) infections in patients with prior hospitalizations (75.1%), indwelling devices (72.8%), and patients discharged to long-term care settings (55.9%); deaths occurred in (9.0%) of these cases (Guh et al., 2015). In a 2012 report of National Nosocomial Infection Surveillance system (NNIS) the proportion of CRE increased from 1.2% in 2001 to 4.2% in 2011, notably observed in *Klebsiella* species (Centers for Disease, Control Prevention, 2013).

Estimates of incidence of various resistance patterns direct appropriate stewardship interventions and infection control measures (Barlam et al., 2016). Applying stewardship interventions in Bispebjerg Hospital in Denmark resulted in a marked reduction in infections caused by ESBL/AmpC-producing bacterial pathogens among inpatients ≥60 years old from a baseline value of 51% in 2009 to 26% in 2011. A parallel reduction was observed in the 30-day mortality rate of patients with ESBL-producing *K. pneumoniae* infections from 35% to 17% (Knudsen et al., 2014). The fact that irrational antibiotics prescribing is one of the most common causes of decreased antibiotics effect toward microorganisms, provides a great opportunity for improvement if we could adopt an evidence-based antimicrobial stewardship program (ASP) dependent on corrective strategies for inappropriate use of antibiotics (Spellberg et al., 2008).

ASP is a multidisciplinary practice adopted by healthcare facilities that focuses on ensuring the proper use of antimicrobials to provide the best patient outcomes, lessen the risk of adverse effects, promote cost-effectiveness, and reduce or stabilize levels of resistance (Owens, 2008). Implementing such programs to improve antibiotic prescribing practices for inpatients involves persuasive (education, audit/feedback, guidelines and clinical pathways), restrictive (formulary restriction, pre-authorization from infectious diseases specialist, automatic stop orders, antimicrobial cycling or scheduled switch, antibiotic order forms) and structural programs (computerized records and decision support interventions) (Davey et al., 2013). Targeted interventions are needed to attain antimicrobial stewardship objectives. For this purpose, we conducted this retrospective review of microbiological data in our institution to evaluate the pattern and prevalence of bacterial resistance and design the hospital antibiogram.

Methods

Study population

Ethical approval was obtained from Suhar Hospital research committee, Ministry of Health, Sultanate of Oman. This retrospective, descriptive, single-center study was conducted at Suhar Hospital, a 450-bed tertiary care facility serving patients of northern Oman, within 3 medical and 7 surgical wards in addition to 4 critical care units and a dialysis unit. We surveyed a convenience sample of all bacterial isolates collected by or referred to the central microbiology laboratory of the hospital between January 1st, 2016 and December 31st, 2017.

Study data acquired from the electronic medical records and microbiology laboratory database included patient demographics (age, gender, nationality,), dates of interest (admission, discharge, sample request, sample collection and release), sample description (requesting location, specimen type, original infection), isolate identification, susceptibility, and resistance phenotype. Samples were excluded per protocol if they fulfil all the following criteria: linked to same patient identification number, collected within less than a week, and identified the same pathogen with identical antibiotic susceptibility pattern.

Identification of microorganisms

All procedures are performed according to Clinical and laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2013). Isolated pathogens were identified based on colony morphology and Gram stain. Gram negative bacilli were further identified based on lactose fermentation; lactose fermenter bacteria were identified using API 20 E kits (BioMérieux Vitek, Inc., USA) while API 20 NE kits (BioMérieux Vitek, Inc., USA) were used to identify the non-lactose fermenter. On the other hand, Gram positive bacteria were further identified based on catalase, coagulase tests and API STAPH kit (BioMérieux Vitek, Inc., USA). For antibiotic susceptibility of the isolated bacteria, Kirby-Bauer disk diffusion technique was used. This method was done on Mueller-Hinton agar (BioOman, Oman) and Mueller-Hinton with blood agar (BioOman, Oman) for streptococci and enterococcus.

All Gram-negative bacteria that were non-susceptible to at least one antibiotic in three or more antimicrobial categories were reported as multidrug resistant (MDR). All *E. coli*, *Proteus* spp. and *Klebsiella* spp. were tested for resistance to 3rd generation cephalosporin antibiotics. The resistant strains were tested for ESBL production by double disc diffusion method (primary test) and then combination disc method (confirmatory test). For the confirmatory test, both cefotaxime and ceftazidime, alone and in combination with clavulanic acid (10 μg) were tested. The criteria for classifying as an ESBL-producing isolate was based on ≥5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid, as opposed to its zone when tested alone. ESBL confirmation is indicated for strains that showed zones of inhibition around: Ceftazidime of ≤22 mm and Cefotaxime ≤27 mm or ceftriaxone of ≤25 mm. All confirmed ESBL producing strains were reported as resistant for all penicillins, cephalosporins (1st, 2nd and 3rd generation cephalosporins) and aztreonam. *E. coli* ATCC (25922) and *K. pneumoniae* ATCC 700604 (obtained from Central Public Health Laboratory -CPHL- of Oman) were used as ESBL negative and ESBL positive control respectively.

All *Enterobacteriaceae* that showed zones of inhibition <23 mm around (Ertapenem 10 μg or Meropenem 10 μg) and usually tested resistant to one or more agents in cephalosporin subclass III (cefotaxime, ceftazidime and ceftriaxone) were considered as CRE. Positive isolates were sent to the national central laboratory

(CPHL) for confirmation using a Modified Hodge Test (Amjad et al., 2011). All confirmed CRE producing strains were reported as resistant for all penicillins, cephalosporins, carbapenems and aztreonam.

In most staphylococcal isolates, oxacillin resistance is mediated by mecA, encoding the penicillin-binding protein 2a (PBP 2a, also called PBP2′). Cefoxitin is used as a surrogate for oxacillin; oxacillin is reported as susceptible or resistant based on the cefoxitin result. Staphylococci that showed zones of inhibition <21 mm around (cefoxitin 30 μg), oxacillin MIC ≥ 4 or cefoxitin MIC ≥ 8 were reported as methicillin resistant (MRSA) in this study. All confirmed MRSA were reported as resistant to all currently available β-lactam antimicrobial agents, with the exception of the newer cephalosporins with anti-MRSA activity. *S. aureus* ATCC 25923 – mecA negative (inhibition zone 23–29 mm) were used as a control organism. Vancomycin resistance enterococci (VRE) were identified based on MIC value of vancomycin after 24 h incubation. All Enterococci isolates for which the vancomycin MICs ≥8 μg/mL were sent to CPHL for VRE confirmation.

Statistical analysis

All statistical analyses were performed using Statistical Analysis System (SAS) version 9.4 (SAS Institute, Cary, NC). Quantitative data are reported as Mean ± Standard Deviation and qualitative data are reported as Counts (Percentage).

Results

A total of 15,733 isolates originated from positive cultures representing 9,231 unique patients, of which 2,434 were patients with multiple cultures (number of cultures range 2–58).

Table 1
Characteristics of patients and study isolates.

Patients Characteristic (n = 9231)		Value
Sex, n (%)		
	Female	5698 (61.72)
	Male	3533 (38.28)
Age, years		
	Mean ± SD	35.19 ± 24.80
Age group, n (%)		
	Infants (0–2 years)	1272 (13.78)
	Children (3–12 years)	720 (7.80)
	Adolescent (13–17 years)	296 (3.21)
	Adults (18–65 years)	5438 (58.91)
	Older adults – Seniors (65+)	1505 (16.30)
Nationality, n (%)		
	Omani	8764 (94.94)
	Other	467 (5.06)
Isolates Characteristic (n = 15,733)		Value
Patient category, n (%)		
	Outpatient (referral & other)	9576 (60.87)
	Inpatient	6155 (39.13)
Request location, n (%)		
	Surgical wards Sohar Hosp.	4277 (27.19)
	Critical care area	4101 (26.07)
	Medical wards sohar Hosp.	3860 (24.54)
	Subordinate Polyclinics	2729 (17.35)
	Subordinate health centers	764 (4.86)
Sample Source, n (%)		
	Skin & Soft tissues	7080 (45.01)
	Urine	4701 (29.88)
	Respiratory system	2228 (14.16)
	Blood	1415 (8.99)
	Patient related devices	143 (0.91)
	Stool	116 (0.74)
	Other Body Fluids	37 (0.24)
	CSF[a]	11 (0.07)

[a] Cerebrospinal fluid.

Table 1 shows the characteristics of the study isolate population. Isolates were predominantly Gram-negative organisms 10,659 (67.7%) while Gram-positive organisms resemble 4,555 (29.0%), Candida fungal infections were 415 cases, while other fungal infections were 41 cases only. Most frequent isolates of Gram-negative and positive organisms were principally obtained from urine and soft tissue samples, collected in surgical wards and critical care areas. Organisms stratified by sample type and request location are detailed in Table 2. Carbapenem, colistin, tigecycline and vancomycin maintained relatively appreciable activity; detailed susceptibilities of most frequent Gram-negative and positive isolates are shown in Figures 1 and 2, respectively. We documented a remarkable number of MRSA isolates as well as other resistant phenotypes as depicted in Figure 3.

Discussion

In this analysis, the infection epidemiology and antimicrobial susceptibility pattern of isolates collected over 2 years were studied, in order to provide accurate information about the bacterial phenotypes as well as its response to therapy, which can be used to rationalize ASP initiatives. The majority of isolates were Gram-negative (67.7%), of which *E. coli* (32.4%) was the most frequent; a similar pattern was observed in 4-year multicenter studies (SENTRY) in Europe and Latin America (20%), (17.2%) respectively (Amjad et al., 2011; Fluit et al., 2000). ESBL producers, *E. coli* and *K. pneumoniae* constituted 37% and 23% of both organisms, respectively. A 2005 Turkish study showed a prevalence of 21% ESBL production among *E. coli* causing community acquired urinary tract infection (UTI) (Sader et al., 2002), whereas it ranged from 13% to 27% for *K. pneumoniae* in studies performed in Spain and the United Kingdom (UK) (Yumuk et al., 2008; Coque et al., 2008).

In our study, ESBL producing *E. coli* and *K. pneumoniae* were mainly collected from urinary tract, skin and soft tissue infections (66%, 25% and 46%, 31%, respectively). ESBL producing *E. coli* were imipenem 99%, amikacin 97%, nitrofurantoin and piperacillin/tazobactam (95%), gentamycin and meropenem (80%) susceptible, but mostly resistant to amoxicillin/clavulanate and ciprofloxacin, while ESBL producing *K. pneumoniae* were tigecycline 100%, imipenem 99%, amikacin 93%, gentamycin (55%), nitrofurantoin (72%), ciprofloxacin (75%) and piperacillin/tazobactam (80%) susceptible. ESBL production was rare in *P. aeruginosa* (2/2362 cases), while no ESBL producing *A. baumannii* was isolated.

CRE was common among *K. pneumoniae* isolates 23.1% compared with *E. coli* 1.3% and *A. baumannii* 0.2%. CRE *K. pneumoniae* were mainly isolated from soft tissue and urinary tract samples collected from critical care areas and medical wards. It showed susceptibility to colistin 93%, and tigecycline 75%, while resistance to all other tested antibiotics was observed. Our findings are consistent with the results observed in different Asian countries based on a systematic review of articles published from January 2001 to December 2013 (Rodriguez-Bano et al., 2006).

With respect to Gram-positive organisms, MSSA, MRSA, CoNS, *S. agalactiae* were the most commonly occurring isolates with percentages 27.2%, 16.1%, 11.5%, 25.4% (out of 4555 total count). MSSA was the most abundant Gram-positive pathogen with a percentage slightly higher than that observed in SENTRY antimicrobial surveillance program (17.6% European arm) (Amjad et al., 2011) and (21.3% Latin American arm) (Fluit et al., 2000). MSSA isolates originated mainly from skin and soft tissue cultures and showed remarkable susceptibility towards cephalexin 100%, amoxicillin/clavulanate 100%, gentamycin 92%, clindamycin 92% and co-trimoxazole, (79%) to ciprofloxacin. On the other hand, Northern Oman appears to have a marked incidence of *MRSA* (37% of S. aureus isolates) as is the case of other countries in the middle

Table 2
Organism types stratified by sample type/request location (n = 15,733).

Sample	Gram-negative n = 10659		Gram-positive n = 4555		Candida n = 415		*Mycobacterium tuberculosis* n = 63		Other Fungi n = 41	
Skin & Soft tissue	4085	38.3%	2854	62.7%	222	53.6%	0	0.0%	37	90.2%
Blood	568	5.3%	837	18.4%	10	2.4%	0	0.0%	0	0.0%
Resp. system	1724	16.2%	267	5.9%	169	40.8%	63	100.0%	4	9.8%
Urine	4146	38.9%	547	12.0%	9	2.2%	0	0.0%	0	0.0%
Patient devices	106	1.0%	33	0.7%	5	1.0%	0	0.0%	0	0.0%
Other Fluids	22	0.2%	14	0.3%	0	0.0%	0	0.0%	0	0.0%
CSF	8	0.1%	3	0.1%	0	0.0%	0	0.0%	0	0.0%
Critical Care	2868	26.9%	1125	24.7%	73	8.9%	35	55.6%	5	12.2%
Medical Wards	2740	25.7%	760	16.7%	94	22.7%	15	23.8%	2	4.9%
Surgical Wards	2856	26.8%	1518	33.3%	129	31.2%	13	20.6%	14	34.1%
Affiliated PC[a]	1560	14.6%	1037	22.8%	111	26.6%	0	0.0%	16	39.0%
Affiliated HC[b]	635	6.0%	115	2.5%	8	1.9%	0	0.0%	4	9.8%

[a] Polyclinics.
[b] Health centers.

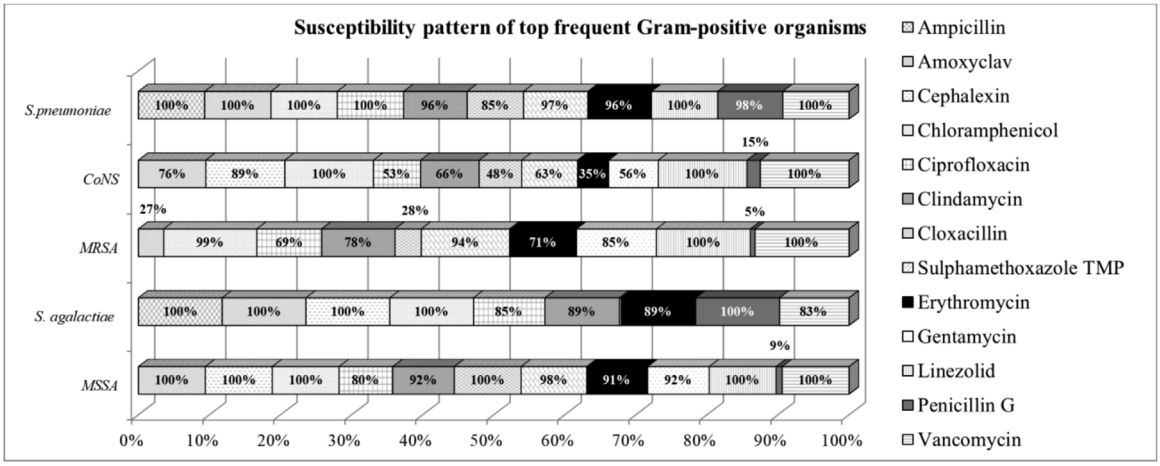

Figure 1. Susceptibility pattern of frequent Gram-positive organisms.
* MSSA = Methicillin Sensitive *Staphylococcus aureus*.
* MRSA = Methicillin resistant *Staphylococcus aureus*.
* CoNS = Staphylococcus, coagulase negative.
* *S. agalactiae* = *Streptococcus agalactiae*.
* *S. pneumoniae* = *Streptococcus pneumoniae*.

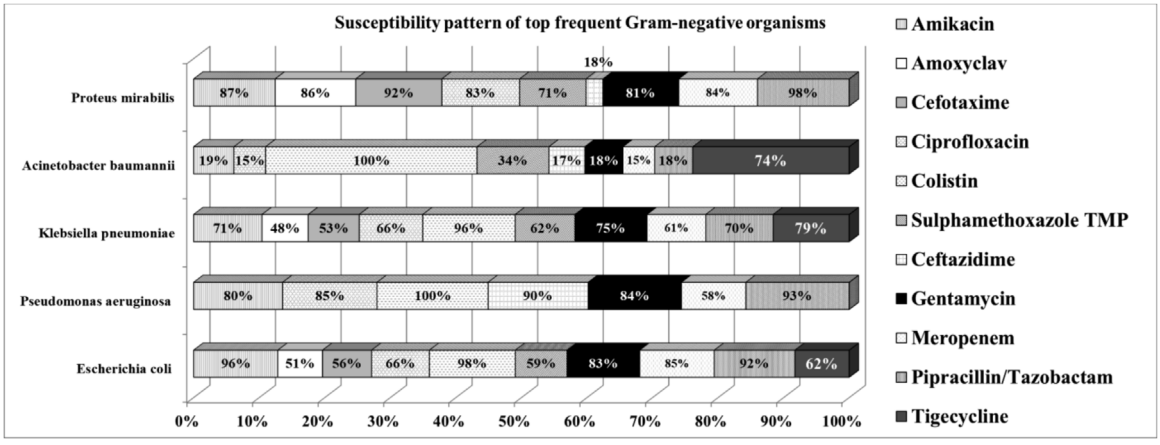

Figure 2. Susceptibility pattern of frequent Gram-negative organisms.

east region; a 3-year study covering 62 hospitals in the middle east region reported percentages ranging from (27.1% to 51.1%) in Algeria, Cyprus, Egypt, Jordan, Lebanon, Malta, Morocco, Tunisia and Turkey (Xu et al., 2015).

Staphylococci, coagulase negative isolates – mainly blood stream infections isolated in critical care areas and medical wards – constitute 11.5% of Gram-positive isolates, which is relatively less than found in Brazil and Jamaica (22%) (Borg et al., 2007; Keim

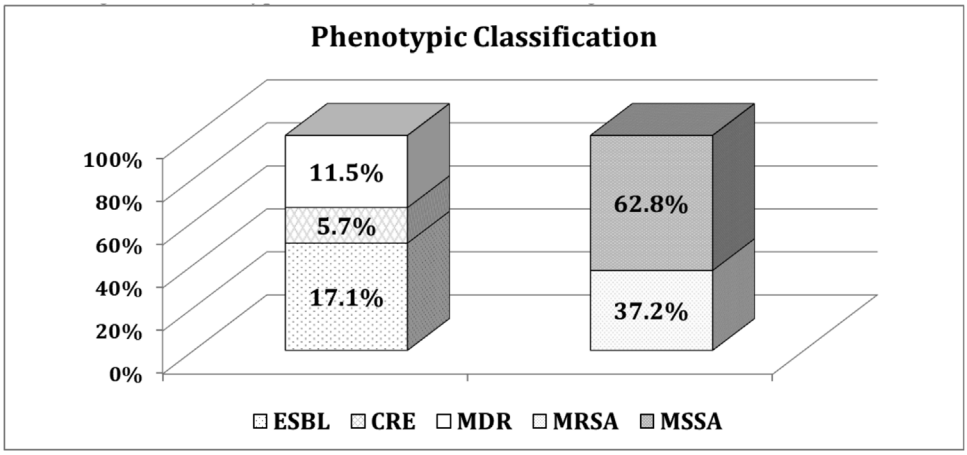

Figure 3. Phenotypic classification of isolated organisms.
** Percentages of ESBL, CRE and MDR are relevant to total gram-negative isolates.
** Percentages of MSSA and MRSA are relevant to total *Staphylococcus aureus* isolates.
* MSSA = Methicillin Sensitive *Staphylococcus aureus*.
* MRSA = Methicillin resistant *Staphylococcus aureus*.
* MDR = Multiple Drug Resistant.
* CRE = Carbapenem-Resistant Enterobacteriaceae.
* ESBL = Extended Spectrum Beta-Lactamase producers.

et al., 2011), and 12% in Ethiopia (Akpaka et al., 2006). It was 100% susceptible to vancomycin and linezolid, 6084% susceptible to co-trimoxazole, clindamycin, amoxicillin/clavulanate and cephalexin, with noticeable resistance to ciprofloxacin (47%), cloxacillin (44%), and erythromycin (33%).

S. agalactiae isolated mainly in the Obstetrics and Gynecology department from vaginal swabs constitutes 25.4% of gram-positive isolates, comparable to what was found in Italy (Deyno et al., 2018), in Taiwan it was a range of 13% in non-pregnant compared to 22% in pregnant females (Matani et al., 2016). It retains 100% of its sensitivity to commonly prescribed Beta-lactams (ampicillin, cephalexin, amoxicillin/clavulanate, penicillin G), vancomycin 83%, ciprofloxacin 86%, clindamycin 88%, erythromycin 88% and linezolid 94%. This approximates the Taiwan study and favorably exceeds the Italian one (Deyno et al., 2018; Matani et al., 2016).

Streptococcus pneumoniae (2.9% of Gram-positive isolates) was infrequently isolated from respiratory tract infections (1%); which are much lower than rates found in studies conducted in other regions of the world, 14.7% and 21% in Malaysia and Belgium respectively (Lee and Lai, 2015; Bahtar et al., 2016). It showed good susceptibility to commonly used β-lactams (ampicillin 100%, penicillin G 96%, amoxicillin/clavulanate 100%, cefotaxime 100%) and 97% towards fluoroquinolones and clindamycin.

Of note, the resistance pattern of *Enterococcal* species was not sufficiently represented because of an inadequate number of isolates. In addition, describing the fungal and mycobacterial resistance patterns were outside the scope of this endeavor.

To our knowledge, this is the first large scale surveillance study in the Sultanate of Oman, however it is limited by a lack of reporting of actual MIC values and lack of genotyping due to retrospective design of data collection, which will be addressed in the prospective phase of this project. Finally, a review covering the period 1990–2011 in 6 gulf countries reported that 37,295 bacterial isolates were studied for antimicrobial resistance. *E. coli* predominates (by 44%), followed by *K. pneumoniae* (20%), *P. aeruginosa* (18.7%), MRSA (5.4%), Acinetobacter (5%). ESBL phenotypes were detected in more than 21% of the total gram-negative isolates (Malfroot et al., 2004). The highest incidence of ESBL was in Qatar showing the following prevalence: ESBL *E. coli* (34%), followed by *Klebsiella* spp. (13.7%) and finally *P. aeruginosa* (7.4%) (Aly and Balkhy, 2012), while MRSA and *S. pneumoniae* showed the highest prevalence in Saudi Arabia with percentages (29.9%) and (30.7%) respectively (Malfroot et al., 2004; Khan et al., 2010).

Conclusion

Based on the results of this study, several recommendations can be made; highly resistant (ESBL & CRE) *E. coli* and *K. pneumoniae* are significant pathogens in bloodstream infections, and empirical treatment options should be revised, drug-bug match therapy instituted promptly and newer agents considered. Gram-negative isolates showed very rare resistance to colistin and tigecycline; thus, in order to maintain efficacy, prescription should be restricted to specialists. Resistance to daptomycin and linezolid was virtually non-existent among Gram-positives, therefore they should only be prescribed subsequent to susceptibility test results. Increased resistance to ciprofloxacin and meropenem warrants review of its use as empiric therapy. Almost 50% of Staphylococcus isolates (MRSA and CoNS) require vancomycin treatment, hence individualized dosing protocol, pharmacokinetic guided monitoring and laboratory surveillance of MICs are warranted in order to increase treatment success rate. The findings from this study (phase I) will guide formulary restriction, selection of empiric therapy, review of treatment protocols (phase II), and finally prospective evaluation of outcomes (phase III) as part of a comprehensive program aiming to establish a functional ASP program.

Conflict of interest

All authors declare no conflict of interest.

Ethical approval

Protocol and data collection approval to was obtained prior initiation of the study from research committee, Sohar Hospital, Ministry of Health, Sultanate of Oman.

Source of funding

There was no funding for the study.

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