Febrile illness of bacterial etiology in a public fever hospital in Egypt: High burden of multidrug resistance and WHO priority Gram negative pathogens

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Abstract

Introduction Contemporary emergence of multidrug resistance (MDR) urges regular updates on circulating pathogens and their antimicrobial resistance profiles. We aimed to identify the burden of MDR and World Health Organization (WHO) priority Gram negative pathogens among patients admitted with febrile illness to Abbassia Fever Hospital, a major Public Fever Hospital in Egypt. The carbapenemase- and extended spectrum beta-lactamases (ESBLs)-encoding genes carried by the isolates were also identified.

Methods A total of 9602 clinical specimens were collected from febrile patients during 2018 and 2019. The recovered bacterial isolates were examined for antimicrobial susceptibility using disk diffusion test. Susceptibility to colistin was tested using E-test. ESBLs production was phenotypically and genotypically analyzed.

Results A total of 790 bacterial isolates (612 Gram negative and 178 Gram positive) were recovered. A percentage of 77.6%, and 62.9% of the Gram negative and positive isolates showed MDR phenotype, respectively. WHO priority pathogens were abundant, including carbapenem-resistant (CR) Enterobacterales (105/187; 56.1%) and CR glucose non-fermenters (82/187; 43.8%) such as: *A. baumannii* (55; 29.4%), *P. aeruginosa* (27; 14.4%). Carbapenemase- and ESBLs-encoding genes were detected in 56.1% and 30.8% of Enterobacterales and in 43.8% and 46.3% of glucose non-fermenters, respectively. Antimicrobials such as fosfomycin and chloramphenicol retained good activities against MDR Gram negative pathogens.

Conclusions This study highlights the regional burden of MDR and priority Gram negative pathogens. The obtained data are of relevant medical importance for implementation of evidence-based antimicrobial stewardship programs and for tailoring the existing empirical treatment guidelines.

Keywords Febrile illness, Gram negative, multidrug resistance, WHO priority pathogens, ESBLs.

Introduction

Fever is one of the common drivers for seeking healthcare in most settings. While non-infectious etiologies may stand behind,[1] fever remains a hallmark sign of many infectious diseases.[2] For successful management of febrile cases, appropriate diagnosis is a must. This is hampered by the limited diagnostic resources and epidemiologic data on common etiologies of fever in low- and middle-income countries. While correct antimicrobial therapy is particularly critical for management of bacterial infections, the emergence and widespread dissemination of multidrug resistance (MDR) has become a global

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crisis.[3,4] Due to the limited therapeutic options with only parenteral administration, high cost and more toxic alternatives, a great challenge is being faced by clinicians for management of MDR infections.[3] Higher length of stay and its associated cost and sometimes mortality are thus becoming imminent.[3] In response to the widespread dissemination of antimicrobial resistance, early in 2017 a list of antibiotic-resistant priority pathogens, for which novel antimicrobial agents are urgently required, was published by the World Health Organization (WHO). In particular, the threat of MDR Gram negative pathogens was highlighted. In their list, the WHO categorized pathogens into critical, high, and medium priority.[4] The critical priority pathogens list included carbapenem-resistant (CR) *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. In addition, extended-spectrum β–lactamase (ESBL)-producers and CR Enterobacterales were also included.

To allow appropriate and timely interventions for febrile patients in the era of disseminated MDR, regional bacterial etiology of febrile illness and their antimicrobial susceptibility patterns should be regularly revised, and evidence-based empirical treatment guidelines are to be developed accordingly. The bacterial spectrum of febrile illness has been documented in Egypt by older studies.[5,6] Nevertheless, these have not been linked to their susceptibility profiles. Moreover, the rising antimicrobial resistance among MDR, extensively drug-resistant (XDR), CR Gram negative isolates have continuously been reported in Egypt.[7,8] However, wide scale studies on the prevalence of MDR as well as priority pathogens are still lacking.

Therefore, this study aimed to identify the common bacterial causes of acute febrile illness in febrile patients admitted to one of the largest public infectious disease hospitals in Egypt during a two-year period (2018-2019). Their antimicrobial susceptibility profiles and the prevalence of carbapenem- and extended spectrum beta-lactamases (ESBLs)-encoding genes and the burden of MDR and WHO Gram negative priority pathogens were also assessed.

Methods

Study design

This is a prospective study performed at Abbassia Fever Hospital in Cairo in the period from January 2018 to December 2019. The Abbassia Fever Hospital is one of the largest infectious disease hospitals in Egypt affiliated to the Egyptian Ministry of Health to which all febrile patients from the Greater Cairo Area are referred. The study protocol was reviewed and approved by the institutional ethics committee, Faculty of Pharmacy, Ain Shams University (ENREC-ASU-2019-268) and the study was conducted in accordance with the Declaration of Helsinki ethical principles.

Microbiological procedures

During the study period, about 9602 clinical specimens were received by the microbiology laboratory of the hospital for investigating the bacterial etiology of acute febrile illness. The isolates were recovered from various clinical specimens obtained from 3203 febrile neutrophilic patients (>11,000 white blood cells/μL with oral temperature >38°C, over at least 1 hour). Patients were excluded if they received inappropriate antibiotic treatment before performing culture and sensitivity testing, and so were cases that gave negative bacterial culture. Positive bacterial cultures were obtained from 790 cases who were selected for enrollment in our study. Standard biochemical and microbiological diagnostics were routinely used for the identification of the positive cultures, as described before.[9] The identification of the recovered isolates was confirmed using the automated microbial identification system, Vitek-2 (bioMérieux, France).

Antimicrobial susceptibility testing

The bacterial isolates were examined for susceptibility to the antimicrobial agents recommended by the Clinical and Laboratory Standards Institute (CLSI, 2018) for each bacterial species.[10] Susceptibility tests were performed using the Kirby-Bauer disk diffusion method on Müller-Hinton agar (Hi media, India) using the following antimicrobial disks (Bioanalyse, Turkey): ampicillin (AM, 10 μg),

amoxicillin/clavulanic acid (AMC, 20/10 µg), ampicillin/sulbactam (SAM, 10/10 µg), piperacillin/tazobactam (TPZ, 10/100 µg), cefaclor (CEC, 30 µg), cefepime (FEP, 30 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg), aztreonam (AZT, 30 µg), imipenem (IMP, 10 µg), meropenem (MEM, 10 µg), ertapenem (ETP, 10 µg), gentamicin (CN, 10 µg and 120 µg) amikacin (AK, 30 µg), trimethoprim-sulfamethoxazole (cotrimoxazole) (COT, 1.25/23.75 µg), ciprofloxacin (CIP, 5 µg), levofloxacin (LEV, 5 µg), norfloxacin (NOR, 10 µg), ofloxacin (OFX, 5 µg), tetracycline (TE, 30 µg), doxycycline (DO, 30 µg), tigecycline (TGC, 15 µg), fosfomycin (FF, 50 µg), chloramphenicol (C, 30 µg). Susceptibility to colistin was examined using E-test (Bioanalyse, Turkey) according to manufacturer's recommendations. Quality control was monitored by using *E. coli* ATCC 25922 reference strain.

Identification of MDR phenotypes

MDR phenotype was inferred as described by Magiorakos et al.[11] whose definition was adopted from those of the European Centre for Disease Prevention and Control and the Centers for Disease Control and Prevention.[11] They defined MDR phenotype as acquired non-susceptibility to at least one agent in three or more antimicrobial categories.[11]

Phenotypic detection of ESBL producers

Rapid phenotypic detection of ESBL producers was carried out using ESBL producers-AmpC kits (Alifax, Italy).

Genotypic detection of ESBL and carbapenemase-encoding genes

Genotypic detection of ESBL and carbapenemase-encoding genes was carried out using PCR as previously described.[12] Chromosomal DNA extraction and the annealing temperatures adjustment for the used primers were undertaken as previously reported.[12] The PCR amplicons were analyzed using agarose gel electrophoresis.[13]

Statistical analyses

Nominal data was expressed in the form of frequency (percentage). Distribution of numerical data was assessed using Shapiro Wilk test. The central tendency of non-parametric data was described by the median. Mann-Whitney U and Kruskal-Wallis tests were used for group comparisons of numerical data not normally distributed. All tests were 2-tailed and *P*-values less than 0.05 were considered as statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp., USA).

Results

Bacterial pathogens causing febrile illness

A total of 790 patients were enrolled in the current study. The patients were admitted to Abbassia Fever Hospital for investigation of acute fever during 2018 and 2019. The mean age of the enrolled participants (n=790) was 49.2±14.5 years, and 67.4% were male. One positive bacterial culture per patient was investigated. Positive bacterial cultures were most commonly obtained from urine specimens (n=340, 43.0%) followed by blood (n=206, 26.1%), sputum (n=115, 14.6%), and cerebrospinal fluid (CSF) (n=93, 11.7%), while stool specimens had the least frequency of isolation of pathogenic bacteria (n=36, 4.6%). Using the identification techniques, 612 Gram negative isolates (77.5%) and 178 Gram positive isolates (22.5%) were identified. *Escherichia coli* and *Klebsiella pneumoniae* predominated among the recovered isolates. The types, number and percentage of clinical samples as well as the age and sex distribution of febrile patients admitted to Abbasia Fever Hospital during the study period is presented in Table 1. The whole spectrum of Gram negative bacterial etiologies of the acute febrile illness in the group of patients enrolled in the current study is shown in Table 2.

Antimicrobial susceptibility findings

The frequency of resistance of different Gram negative species to the tested antimicrobial agents is presented in Figure 1 and Table 3. Only one *Citrobacter* isolate was identified in the

Table 1. Types and number of samples and age and sex distribution of febrile patients admitted to Abbasia Fever Hospital during the study period

Type of Sample	Total samples (n=9602)			Positive samples (n=790)		
	Number (%)	Mean age ± SD, years	Male sex, n (%)	Number (%)	Mean age ± SD, years	Male sex, n (%)
Urine	4096 (42.6%)		1925 (46.9%)	340 (43.0%)		146 (42.9%)
Blood	2575 (26.8%)		1987 (77.1%)	206 (26.1%)		194 (94.2%)
Sputum	1402 (14.7%)	45±12.5	760 (54.2%)	115 (14.6%)	49.2±14.5	97 (84.3%)
CSF	1240 (12.9%)		650 (52.4%)	93 (11.7%)		64 (68.8%)
Stool	289 (3.0%)		260 (89.9%)	36 (4.6%)		32 (88.8%)
Total	9602 (100%)		5582 (58.1%)	790 (100%)		533 (67.4%)

CSF – cerebrospinal fluid; **SD** – standard deviation.

Table 2. The spectrum of Gram negative species isolated from different clinical specimens recovered from febrile patients admitted to Abbasia Fever Hospital during the study period

Bacterial species	Total (n=790)	Blood (n=206)	CSF (n=93)	Sputum (n=115)	Stool (n=36)	Urine (n=340)
Gram negative bacteria	612 (77.4%)					
E. coli	233 (29.4%)	21 (9.0%)	8 (3.4%)	9 (3.9%)	0 (0.0%)	195 (83.7%)
K. pneumoniae	212 (26.8%)	29 (13.7%)	18 (8.5%)	65 (30.7%)	0 (0.0%)	100 (47.2%)
A. baumannii	55 (6.9%)	28 (50.9%)	8 (14.5%)	14 (25.5%)	0 (0.0%)	5 (9.1%)
P. aeruginosa	38 (4.8%)	4 (10.5%)	11 (28.9%)	16 (42.1%)	0 (0.0%)	7 (18.4%)
Salmonella spp.	33 (4.1%)	1 (3.0%)	0 (0.0%)	0 (0.0%)	32 (97.0%)	0 (0.0%)
Brucella spp.	15 (1.9%)	15 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
E. cloacae	13 (1.6%)	6 (46.2%)	2 (15.4%)	3 (23.1%)	0 (0.0%)	2 (15.4%)
Proteus mirabilis	8 (1.0%)	1 (12.5%)	1 (12.5%)	1 (12.5%)	0 (0.0%)	5 (62.5%)
Shigella dysenteriae	4 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (100.0%)	0 (0.0%)
Citrobacter freundii	1 (0.1%)	1 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

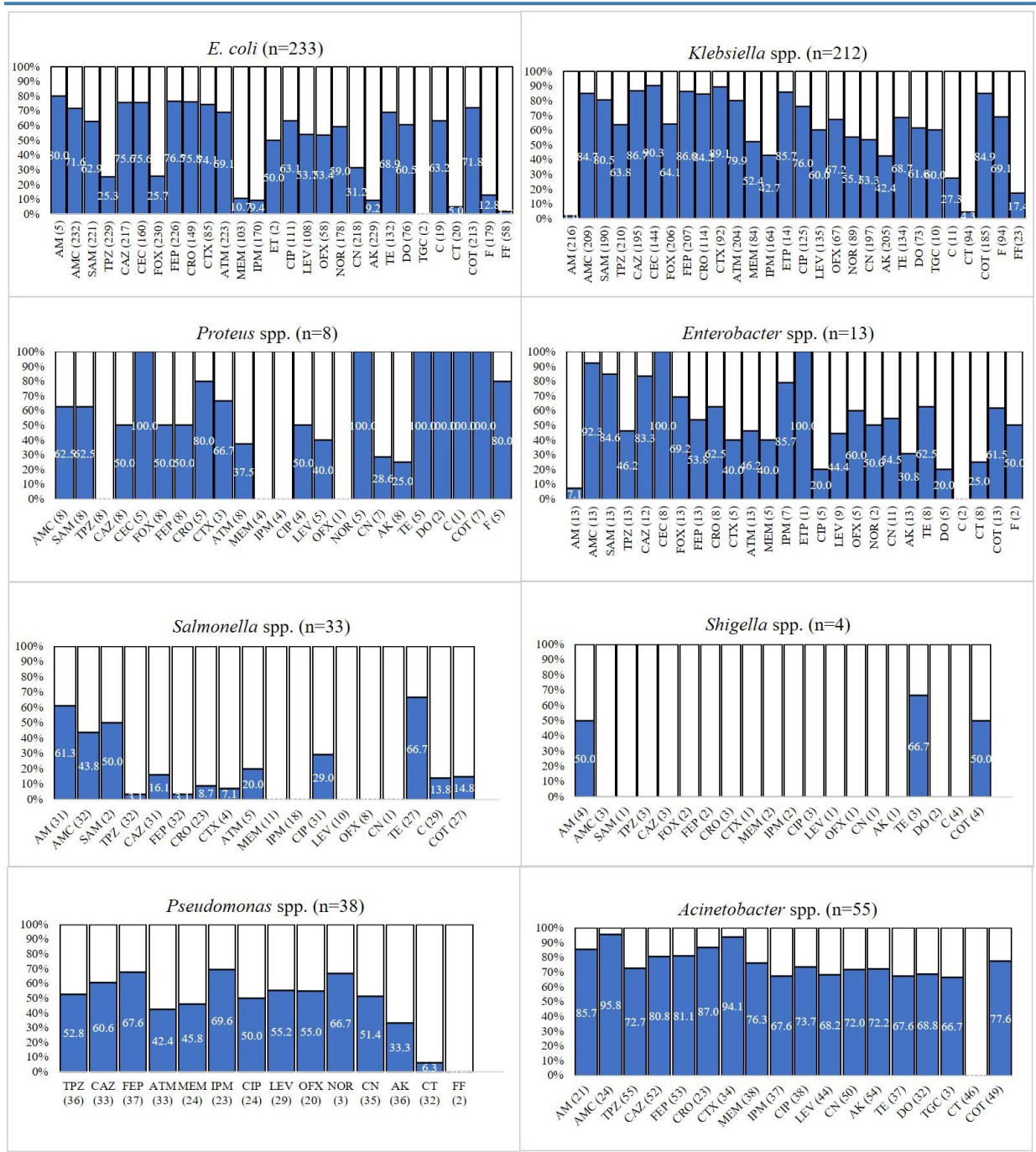Data are presented as n (%) of cultures.
CSF – cerebrospinal fluid.

current study and was found to show non-susceptibility to both ciprofloxacin and amikacin. MDR was identified in 77.6% (475/612) of the Gram negative isolates. Most frequently, MDR phenotype was exhibited by *Proteus* spp. (8/8; 100%), *K. pneumoniae* (193/212; 91.0%), *A. baumannii* (47/55; 85.5%) and *E. coli* (190/233; 81.5%). This was less common among other genera including *Enterobacter*, *Pseudomonas*, *Shigella* and *Salmonella* in the percentages 69.2% (9/13), 65.8% (25/38), 50.0% (2/4) and 3.0% (1/33), respectively.

Antimicrobial susceptibility MDR pathogens

To provide more useful information for guiding empirical treatment and alternative treatment for MDR isolates, the resistance frequency for the tested antimicrobial agents in Gram negative isolates was analyzed (Table 3).

The distribution of the number of antimicrobial classes to which the isolates showed reduced susceptibility in different species was analyzed using Kruskal-Wallis test. The results demonstrated a significant difference in the distribution across the categories of Gram negative bacterial species (p<0.001) as shown in Figure 2. In contrast, this was not found when the results were compared across the two years in which the study was performed (Mann-Whitney U test, p=0.154).

Percentages were calculated with reference to the total number of the tested isolates shown between brackets.
AM – ampicillin; AMC – amoxicillin/clavulanic acid; AK – amikacin; ATM – aztreonam; C – chloramphenicol; CAZ – ceftazidime; CEC – cefaclor; CIP – ciprofloxacin; CN – gentamicin; COT – trimethoprim–sulfamethoxazole; CRO – ceftriaxone; CT – colistin; CTX – cefotaxime; DO – doxycycline; ETP – ertapenem; F – fosfomycin; FEP – cefepime; FF – fosfomycin; FOX – cefoxitin; IPM – imipenem; LEV – levofloxacin; MEM – meropenem; NOR – norfloxacin; OFX – ofloxacin; SAM – ampicillin/sulbactam; TE – tetracycline; TGC – tigecycline; TPZ – piperacillin/tazobactam.

Figure 1. Frequency of antimicrobial resistance of various Gram negative species to the tested antimicrobial agents

Table 3. Resistance frequency of the tested antimicrobial agents in all and MDR Gram negative isolates

Antimicrobial agent	Total Gram negative isolates			MDR Gram negative isolates		
	Total no. tested	R (%)	S (%)	Total no. tested	R (%)	S (%)
Amikacin	547	165 (30.2%)	382 (69.8%)	462	165 (35.7%)	297 (64.3%)
Amoxicillin/clavulanat	522	397 (76.1%)	125 (23.9%)	419	359 (85.7%)	60 (14.3%)
Ampicillin	66	48 (72.7%)	18 (27.3%)	26	25 (96.2%)	1 (3.8%)
Ampicillin/sulbactam	436	309 (70.9%)	127 (29.1%)	369	291 (78.8%)	78 (21.2%)
Aztreonam	487	341 (70.1%)	146 (29.9%)	410	337 (82.2%)	73 (17.8%)
Cefaclor	318	264 (83.1%)	54 (16.9%)	272	254 (93.4%)	18 (6.6%)
Cefepime	580	431 (74.3%)	149 (25.7%)	467	418 (89.5%)	49 (10.5%)
Cefotaxime	234	182 (77.8%)	52 (22.2%)	190	176 (92.6%)	14 (7.4%)
Cefoxitin	460	204 (44.3%)	256 (55.7%)	396	199 (50.3%)	197 (49.7%)
Ceftazidime	552	414 (75.0%)	138 (25.0%)	446	394 (88.3%)	52 (11.7%)
Ceftriaxone	326	240 (73.6%)	86 (26.3%)	254	228 (89.8%)	26 (10.2%)
Chloramphenicol	66	20 (30.3%)	46 (69.7%)	27	17 (62.9%)	10 (37.1%)
Ciprofloxacin	342	218 (63.7%)	124 (36.3%)	265	207 (78.1%)	58 (21.9%)
Colistin	200	9 (4.5%)	191 (95.5%)	170	5 (2.9%)	165 (97.1%)
Cotrimoxazole	499	369 (73.9%)	130 (26.1%)	411	359 (87.3%)	52 (12.7%)
Doxycycline	190	116 (61.1%)	74 (38.9%)	163	116 (71.2%)	47 (28.9%)
Ertapenem	16	13 (81.2%)	3 (18.8%)	15	13 (86.7%)	2 (13.3%)
Fosfomycin	83	5 (6.1%)	78 (93.9%)	66	5 (7.6%)	61 (92.4%)
Gentamicin	521	235 (45.1%)	286 (54.9%)	436	230 (53.8%)	206 (47.2%)
Imipenem	426	128	298 (69.6%)	347	126 (36.3%)	221 (63.7%)
Levofloxacin	342	191 (55.8%)	151 (44.2%)	281	189 (67.3%)	92 (32.7%)
Meropenem	272	97 (35.6%)	175 (64.4%)	211	97 (45.9%)	114 (54.1%)
Nitrofurantoin	280	93 (33.2%)	187 (66.8%)	244	89 (36.5%)	155 (63.5%)
Norfloxacin	277	162 (58.5%)	115 (41.5%)	240	160 (66.7%)	80 (33.3%)
Ofloxacin	160	90 (56.2%)	70 (43.8%)	126	87 (69.1%)	39 (30.9%)
Piperacillin/tazobactam	587	258 (43.9%)	329 (56.1%)	469	256 (54.6%)	213 (45.4%)
Tetracycline	347	238 (68.5%)	109 (31.5%)	275	217 (78.9%)	58 (21.1%)
Tigecycline	15	8 (53.3%)	7 (46.7%)	14	8 (57.1%)	6 (42.9%)

R (%) – number of resistant isolates (percentage); **S** (%) – number of susceptible isolates (percentage); **MDR** – multidrug resistance.

PCR detection of ESBL and carbapenemase-encoding genes

A total of 575 Gram negative isolates were tested for susceptibility to at least one carbapenem antimicrobial agent. Of them, carbapenem resistance was identified in 187 (32.5%) isolates (Table 4). This showed higher prevalence among Enterobacterales isolates (105/187; 56.1%) as compared to glucose-nonfermenters (82/187; 43.8%). Carbapenem resistance was most frequently found in *A. baumannii* (55; 29.4%), *K. pneumoniae* (49; 26.2%). *E. coli* (33/ 17.6%), *P. aeruginosa* (27; 14.4%) and *Enterobacter* (23; 12.3%). None of the isolates that belonged to other species showed non-susceptibility to any of the tested carbapenems. ESBL genes were detected in 30.8% (155/504) of the tested Enterobacterales isolates predominantly in *K. pneumoniae* (52.9%; 82/155), followed by *E. coli* (61/155; 39.4%) and

Enterobacter (12/155; 7.7%). ESBL genes were also detected in 46.3% (38/82) of the tested glucose-non fermenter isolates predominantly in *A. baumannii* (23; 60.5%), followed by *P. aeruginosa* (15; 39.5%) (Table 4).

Discussion

The current study provides an update on the recent spectrum of Gram negative bacterial isolates causing febrile illness and their susceptibility profiles. Reports about MDR, XDR, and CR-Gram negative isolates have been increasingly published from Egypt.[8] However, wide scale studies on the prevalence of MDR as well as priority pathogens are still lacking.

Upon culturing 9602 clinical specimens received by the hospital's laboratory for investigation of acute febrile illness, only 8.2% showed bacterial growth. Several viral, fungal, and parasitic infections may also be implicated.[14] In addition, other non-infectious etiologies of fever may also exist. Among others, neoplastic, vascular, inflammatory, drug-induced causes of fever have been reported.[1] Most commonly, bacterial pathogens were recovered from urine specimens. Urinary tract infection (UTI) is known to be one of the most common infectious diseases.[14] While being mostly benign in healthy adults, UTIs are frequently associated with unfavorable medical sequelae at the extremes of age.[15] Fever mostly reflects invasive UTI such as pyelonephritis, prostatitis or life-threatening urosepsis.[14] UTIs have been identified among the causes of fever and fever of unknown origin in developing countries.[15] In agreement with others,[16-18] *E. coli* and *K. pneumoniae* were the most common of Gram negative uropathogens.

Second to UTIs, bloodstream infections were predominant in febrile cases of bacterial etiology in our study (26.0%). *K. pneumoniae* predominated (3.6% of all positive cultures) followed by *A. baumannii* (3.5%). Low prevalence of other infections known to be associated with febrile bloodstream infection cases in older studies,[5,6] such as brucellosis (1.8%) and salmonellosis (0.1%), was recorded. A possible explanation for this low prevalence is that only culture-based diagnosis was reported here rather than the serological one. It is noteworthy that culture-based diagnosis was confirmed in only 36% of brucellosis cases identified in the studies conducted by Parker et al.[5] and Afifi et al.,[6]
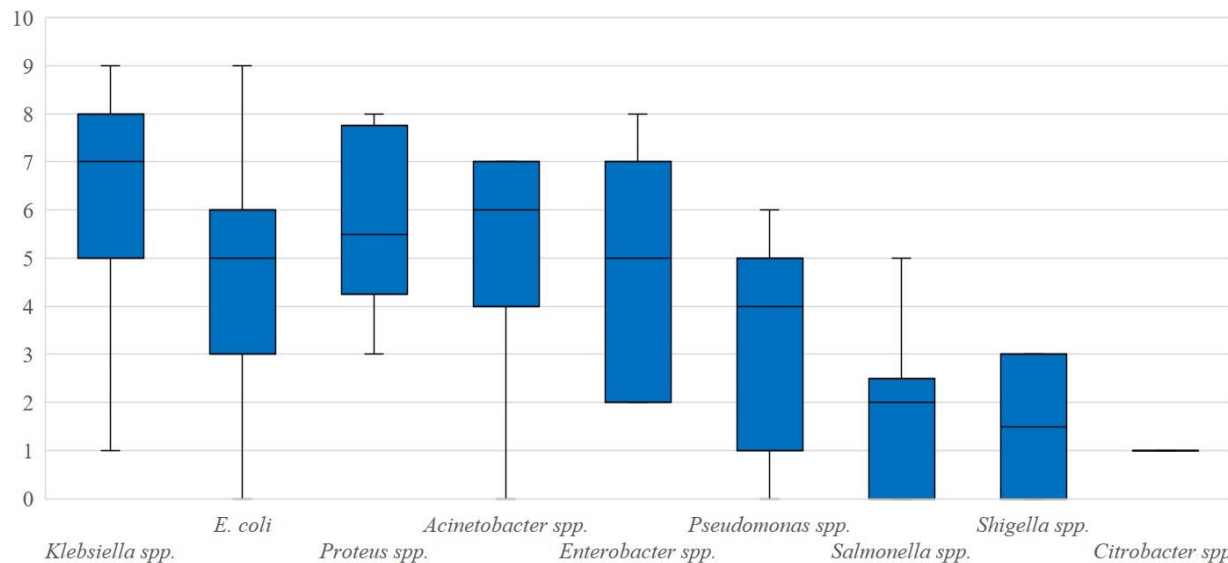
The median is represented by the horizontal line in the box, the lower line of the box is the first quartile, the upper line of the box is the third quartile, and the maximum and minimum data values are represented by the whiskers (the end of the vertical lines).

Figure 2. Box and whisker plot of the number of antimicrobial classes for which isolates of different species were non-susceptible

Table 4. PCR detection of ESBL- and carbapenemase-encoding genes among Enterobacterales and glucose-nonfermenters clinical isolates

Class	Gene	Enterobacterales isolates (n=155/504; 30.8%)			Glucose non-fermenters (n=38/82; 46.3%)	
		E. coli (61; 39.4%)	*K. pneumoniae* (82; 52.9%)	*E. cloacae* (12; 7.7%)	*P. aeruginosa* (15; 39.5%)	*A. baumannii* (23; 60.5%)
ESBLs	bla_{CTX-M}	23 (37.7%)	21 (25.6%)	8 (66.6%)	3 (20%)	5 (21.7%)
	bla_{SHV}	8 (13.1%)	29 (35.3%)	2 (16.6%)	2 (13.3%)	1 (4.3%)
	bla_{TEM}	10 (16.4%)	12 (14.6%)	2 (16.6)	9 (75%)	14 (60.8%)
	bla_{CTX-M}, bla_{SHV}	8 (13.1%)	14 (17.0%)	0 (0%)	0 (0%)	1 (4.3%)
	bla_{CTX-M}, bla_{TEM},	7 (11.4%)	5 (6.1%)	0 (0%)	1 (6.6%)	2 (8.6%)
	bla_{SHV}, bla_{TEM}	5 (8.1%)	1 (1.2%)	0 (0%)	0 (0%)	0 (0%)

Class	Gene	Enterobacterales isolates (n=105/187; 56.1%)			Glucose non-fermenters (n=36/82; 43.9%)	
		E. coli (33/187; 17.6%)	*K. pneumoniae* (49/187; 26.2%)	*E. cloacae* (23/187; 12.3%)	*P. aeruginosa* (27/187; 14.4%)	*A. baumannii* (55/187; 29.4%)
CR	bla_{KPC}	0 (0%)	2 (4.1%)	0 (0%)	0 (0%)	0 (0%)
	bla_{IMP}	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
	bla_{VIM}	5 (15.1%)	5 (10.2%)	1 (4.3%)	6 (22.2%)	16 (29.0%)
	bla_{NDM}	7 (21.2%)	25 (51.0%)	2 (8.6%)	3 (11.1%)	3 (5.4%)
	bla_{OXA}	21 (63.6%)	17 (34.6%)	20 (86.9%)	18 (66.6%)	36 (65.5%)

bla_{CTX-m}: gene coding for cefotaxime (CTX-M) extended-spectrum β-lactamase, bla_{TEM}: gene coding for TEM extended-spectrum β-lactamase, bla_{SHV}: gene coding for SHV extended-spectrum β-lactamase, bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{OXA-48} and bla_{IMP}: gene codes for KPC, NDM, VIM, OXA-48-like, and IMP carbapenemases, respectively.
ESBLs – extended-spectrum β–lactamases; **CR** – carbapenem-resistant.

meanwhile, 64% were identified serologically. Similarly, Afifi et al.[6] have reported enteric fever in 5% of acute febrile illness cases diagnosed by blood cultures and an additional 18% diagnosed serologically. Respiratory tract infections (RTIs) were the third among the bacterial causes of fever (1.4%). In agreement with others,[19] *K. pneumoniae* was the most frequent respiratory pathogen. Less commonly, bacterial meningitis was identified (1.1%) predominantly caused by *S. pneumoniae*. The recent predominance of *S. pneumoniae* as the major cause of bacterial meningitis in Egypt has been previously reported.[9] Driven by the steady rise in antimicrobial resistance, a list of antimicrobial resistant pathogens posing a significant threat to human health was published by the WHO. The organization aimed at encouraging funding the discovery of novel antimicrobial agents to fight the threat of the listed pathogens. Among the high priority pathogens described by the WHO are carbapenem-resistant *A. baumannii* and *P.*

aeruginosa, in addition to ESBL-producers and carbapenem-resistant Enterobacterales. *A. baumannii* is among the highly problematic Gram negative pathogens of which a substantial proportion are carbapenem-resistant. In the current study, this pathogen was isolated from 55 patients. Of them, 85.5% were MDR and 72.7% were resistant to at least one carbapenem antimicrobial agent. Wide dissemination of carbapenem resistant *A baumannii* (CRAB) was also reported regionally[20] and worldwide.[21] Therapeutic options for the treatment of CRAB infections are limited to colistin, tigecycline and some aminoglycosides.[22] Fortunately, neither colistin nor tigecycline resistance were recorded in any of the tested *Acinetobacter* isolates while 90.0% of the carbapenem resistant *Acinetobacter* isolates were non-susceptible to at least one aminoglycoside. MDR and carbapenem-resistance were identified in 65.8% and 71.0% (27/38) of *P. aeruginosa* isolates, respectively. Meanwhile,

colistin resistance was evident in two *Pseudomonas* isolates.

Of 504 Enterobacterales isolates investigated here, 80.0% showed MDR and 24.8% were non-susceptible to at least one carbapenem. The high prevalence of MDR bacteria is in line with published reports about the high prevalence of antibiotics use in Egyptian hospitals and among healthcare practitioners, particularly β-lactam antimicrobial agents.[23,24] MDR was most frequently found in *P. mirabilis* (100%), *K. pneumoniae* (91.0%) and *E. coli* (81.5%) isolates. Interestingly, 46.6% of the carbapenem-resistant Enterobacterales were represented by *K. pneumoniae*. Other carbapenem resistant isolates comprised *E. coli* (31.4%) and *Enterobacter cloacae* (21.9%). In this study, carbapenemase- and ESBLs-encoding genes were detected in 56.1% and 30.8% of Enterobacterales and in 43.8% and 46.3% of glucose non-fermenters, respectively. Carbapenem-resistant *K. pneumoniae* (CRKP) is well-recognized as a leading cause of hospital acquired infections worldwide[25] and in Egypt.[26] An emerging hypervirulent CRKP was also recently reported with expectations to be "the next superbug".[24] ESBL producers were encountered in 30.8% of Enterobacterales isolates. More than half of *K. pneumoniae* isolates were ESBL producers. Similar findings were previously reported.[27]

Due to the emergence of resistant strains, fluoroquinolones and extended spectrum cephalosporins were recommended as alternatives for treatment of invasive *Salmonella* infections.[28] However, the alarmingly rising reports on ciprofloxacin resistance[28] urged the WHO to categorize fluoroquinolone-resistant *Salmonella* among the high priority pathogens calling for novel antimicrobial treatment options. *Salmonella* spp. was isolated from 33 participants in the current study. While only one isolate was non-susceptible to all front-line antimicrobials and hence considered as MDR, 9/31 (29.0%) ciprofloxacin-resistant *Salmonella* isolates were recorded. This was possibly due to widespread use of ciprofloxacin as the drug of choice for treatment of *Salmonella* infections over the last two decades. In support of this finding, re-emerging susceptibility to the front-line antimicrobial agents was also reported elsewhere.[28] In contrast to our findings, higher levels of MDR *Salmonella* were reported by others in Egypt.[29]

Brucella is a zoonotic pathogen affecting livestock causing abortion and infertility.[30] Disease transmission to humans causes undulant fever sometimes associated with chronic complications and mortality in severe or untreated cases.[30] Despite the considerable number of brucellosis cases received by the infectious disease hospitals in Egypt,[6] antimicrobial susceptibility testing is not routinely conducted for *Brucella* isolates. This is in part due to the fastidious growth requirements and the laboratory-acquired infection risk.[30] Accordingly, no data was available for the susceptibility profiles of the *Brucella* isolates recovered in the current study. An empirical treatment of doxycycline combined with either rifampin or streptomycin has been recommended by the WHO.[29] Despite the frequent empirical treatment of brucellosis cases in Egypt, susceptibility to treatment regimens is still preserved.[30]

Taken together, the antimicrobial susceptibility findings of the current study were used for ranking the tested antimicrobial agents based on their activity against all isolates as well as those showing MDR. The top ranked antimicrobials that showed highest activity against Gram negative isolates were colistin (96%), fosfomycin (94%), amikacin (70%), chloramphenicol (70%), and imipenem (70%). Comparable susceptibility (64-100%) was identified to the mentioned antimicrobials in MDR isolates except for chloramphenicol for which only 37% of the tested MDR isolates were susceptible. Our findings support other calls for reviving interest in older antimicrobials for management of infections caused by MDR bacterial strains.[31]

Conclusions

The current study demonstrates the bacterial contributors to acute febrile illness encountered in one of the major infectious disease hospitals in

Egypt. A high burden of MDR and priority pathogens non-susceptible to commonly used antimicrobials was noted among the circulating pathogens. We provided a ranking of the tested antimicrobials with respect to their activity against Gram positive and Gram negative isolates to inform the choice of empirical treatment regimens. Antimicrobials such as nitrofurantoin, fosfomycin and chloramphenicol still retain good antimicrobial activities. Regular publication of updated information from infectious disease hospitals on circulating pathogens and their susceptibility profiles is urgently required. This is expected to enhance the implementation of evidence-based antimicrobial stewardship programs and of tailoring empirical treatment guidelines that perfectly fit our needs.

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