Original Research

MMJ

Antimicrobial susceptibility profiles of clinically important bacterial pathogens at the Kamuzu Central Hospital in Lilongwe, Malawi

Faheema E Choonara[1,2,3], Bjørg C. Haldorsen[4], Isaac Ndhlovu[2], Osborne Saulosi[2], Tarsizio Maida[2], Fanuel Lampiao[3], Gunnar S. Simonsen[4,5], Sabiha Y. Essack[1], and Arnfinn Sundsfjord[4,5]

1. Antimicrobial Research Unit, College of Health Sciences, University of KwaZulu-Natal, Durban, South Africa
2. Microbiology Laboratory, Kamuzu Central Hospital, Lilongwe, Malawi.
3. Africa Centre of Excellence in Public Health and Herbal Medicine, College of Medicine, University of Malawi, Blantyre, Malawi.
4. Department of Microbiology and Infection Control, Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res), University Hospital of North Norway, Tromsø, Norway.
5. Research Group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway.

*Corresponding Authors: Faheema Choonara: faheemachoonara@yahoo.co.uk; Arnfinn Sundsfjord: Arnfinn.sundsfjord@uit.no

Abstract

Background
The aim of this prospective study was to ascertain antimicrobial resistance (AMR) in clinical bacterial pathogens from in-hospital adult patients at a tertiary hospital in Lilongwe, Malawi.

Methods
Clinical specimens (blood culture, pus, urine and cerebrospinal fluid) collected during June to December 2017 were examined for bacterial growth in standard aerobic conditions. One specimen per patient was included. Antimicrobial susceptibility testing (AST) was performed using the disk diffusion method and interpreted according to EUCAST guidelines.

Results
A total of 694 specimens were collected during the study period, of which 336 (48%) specimen yielded visible bacterial growth. Of the 336 specimens, a total of 411 phenotypically different isolates were recovered. Of the 411 isolates, 84 isolates (20%) were excluded and the remaining 327 (80%) were further characterised. The characterised isolates were identified as ESKAPE pathogens (n=195/327; 60%), *Escherichia coli* (n=92/327; 28%), *Proteus mirabilis* (n=33/327; 10) or *Salmonella* spp. (n=7/327; 2%) and were included for further analysis. The excluded isolates (n=84) comprised of coagulase-negative staphylococci (n=25), streptococci (n=33), and low-prevalence Gram-negative bacilli (n=26). *E. coli* (n=92; 28%) and *S. aureus* (n=86; 26%) were the most dominant species. A multidrug resistant (MDR) extended spectrum β- lactamase (ESBL)-positive phenotype was detected in *Klebsiella pneumoniae* (n=20/29; 69%) and *E. coli* (n=49/92; 53%). One third of the *Pseudomonas aeruginosa* isolates were resistant to meropenem (MEM), but did not appear to be carbapenemase-producers. Methicillin resistant *Staphylococcus aureus* (MRSA) was molecularly confirmed in 10.5% of *S. aureus* (n=9/86).

Conclusion
The high proportion of the MDR ESBL-phenotype in clinical isolates of *Enterobacterales*, strongly limits antimicrobial treatment options and has consequences for empirical and targeted antimicrobial treatment as well as clinical microbiology services and hospital infection control. There is need for a continuous surveillance and an antimicrobial stewardship (AMS) program to contain and prevent the spread of AMR.

Keywords; ESKAPE, antimicrobial resistance, ESBL, AmpC

Background

Antimicrobial resistance (AMR) in bacterial pathogens is a global health threat[1-2]. However, the magnitude of the problem is still to be determined in many parts of the world. This is of particular concern in low- and middle-income countries (LMICs), often with a higher burden of infectious diseases, and where antimicrobial treatment guidelines are based on insufficient surveillance data due to limited diagnostic capacity[3-4]. In Malawi, a rapid expansion of extended spectrum β- lactamase (ESBL)-producing *Enterobacterales* and emerging methicillin resistant *Staphylococcus aureus* (MRSA) have been documented in blood cultures isolates on patient admission to Queen Elizabeth Central Hospital, Blantyre[5]. AMR was also reported in various clinical specimens obtained from patients at Kamuzu Central

Hospital, Lilongwe (KCH), on admission during 2006-7[6]. One third of *S.aureus* was resistant to oxacillin, indicating MRSA, and 18.8% of Gram-negative bacteria were resistant to ceftriaxone[6]. In a study of burn patients at KCH in 2015, an increasing rate of wound colonization with multidrug resistant (MDR) *Enterobacterales* and/or MRSA up to 40% and 39%, respectively, were observed during hospitalisation[7]. However, the national AMR-data in Malawi is still limited and it is important to obtain a comprehensive understanding of the problem.

AMR is a particular problem in ESKAPE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) as they are major causes of hospital acquired infections (HAIs) in vulnerable patients[8]. Thus, it is important to

investigate the antimicrobial susceptibility profiles of these pathogens to inform empiric treatment. The aim of the study was to ascertain AMR in clinical ESKAPE-isolates and selected prevalent bacterial pathogens from patients admitted at KCH, a tertiary hospital in Lilongwe, Malawi.

Methods

Study design

A prospective observational study was conducted over a 6 month period from June –December 2017 at KCH, a governmental 750-bed hospital that serves the Lilongwe district and offers referral services to the central region of Malawi, with 1.6 and 7.5 million people, respectively[9]. Microbiology specimens were collected from hospitalised adult patients (>18 years) suspected to have a clinical infection. Specimen types included urine, blood cultures, cerebrospinal fluid (CSF), other sterile fluids, and pus. The clinical staff collected the samples based on the clinical diagnosis of infection made by a physician. Specimens were submitted to the microbiology department in the hospital laboratory for analysis within 24 hours according to specific guidelines (Supplementary Figure 1). The clinical staff were trained in sample and data collection criteria prior to the study. A focal contact person was identified in each department to facilitate study implementation, specimen collection and data reporting.

Data collection and bacterial culture

Patient information was collected using a standardised data form that also served as a specimen request form for submission to the laboratory (Supplementary Figure 2). The collected data did not allow a distinction between community or hospital acquired infection. Data on HIV status of the patients was not collected. Unique identifiers were then allocated to each patient. Data on the overall admissions and the number of patients during the study period were obtained from the hospital's data collection system.

Pus and sterile site fluids (ascites, pleura, knee, and sinus), including positive blood culture samples were inoculated on chocolate agar, Columbia blood agar and MacConkey agar (Oxoid, Hampshire, UK) and incubated for 16- 24h at 35±1°C in ambient air except for chocolate agar which was incubated 16- 24h at 35±1°C in 5% CO^2. For urine specimens, a 1 μl calibrated disposable sterile loop inoculum was cultured on blood agar (Oxoid, Hampshire, UK) and MacConkey agar (Oxoid) at 35±1 0C for 16-20h. Mid-stream urines with $\geq 10^5$ CFU/ml and catheter-/suprapubic urines with $\geq 10^3$ CFU/ml were defined as significant bacteriuria and were selected for microbial culture and antimicrobial susceptibility testing (AST). For blood cultures, approximately 7-10 ml of blood was collected aseptically and inoculated into BD BACTECTM Plus Aerobic medium blood culture bottle (Becton and Dickinson, Franklin, US) and inverted 3-4 times. The bottles were then incubated in the BD BACTECTM 9050 instrument (Becton and Dickinson, Franklin, US) for 5 days. Anaerobic culture was not performed due to the lack of suitable equipment.

Bacteria were identified by Gram stain. Gram-negative bacteria were further identified by the standard oxidase test, and analytical profile index (API) 20E and 20NE systems (BioMerieux, Durham, US) for oxidase negative and positive bacteria, respectively according to manufacturer instructions. Gram-positive bacteria were further identified using the standard catalase test, coagulase test for *Staphylococcus* sp.

and/or streptococcus latex agglutination tests and bile aesculin test for the identification of enterococci. *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as quality control (QC) strains. The QC-strains were used to perform QC on reagents, media and antibiotic discs on a monthly basis and for each new batch of media or reagents. In addition, the laboratory was also enrolled onto the National Heath Laboratory Service external quality assurance scheme for bacteriology. Bacterial isolates identified as ESKAPE pathogens, together with *E. coli, Proteus mirabilis* and *Salmonella* spp. were included in the study. Only one isolate per species per patient was examined.

Antimicrobial susceptibility testing (AST)

AST was performed on Mueller Hinton agar (MAST Diagnostics, Merseyside, UK) by the EUCAST disc diffusion method[10] and interpreted according to EUCAST clinical breakpoints version 4 (http://www.eucast.org/clinical_breakpoints/). Zone inhibition diameters within the R- and I-categories were defined as reduced susceptibility. Isolates were defined as MDR if they were resistant to at least one agent in three or more distinct antimicrobial classes[11]. Selection of antibiotics (MAST) for each bacterial species was based on a combination of the EUCAST recommendations and the availability of antibiotics in the local hospital pharmacy (Supplementary Table 1). For *Enterobacterales*, the antibiotics were ; ampicillin (AMP), aztreonam (AZT), cefotaxime (CTX), cefoxitin (FOX), ceftazidime (CAZ), cefuroxime (CXM), ciprofloxacin (CIP), gentamicin (GEN), meropenem (MEM), piperacillin-tazobactam (TZP), and trimethoprim-sulfamethoxazole (SXT). The *A. baumannii* panel consisted of CIP, GEN, MEM, SXT and the *P. aeruginosa* panel consisted of AZT, CAZ, CIP, MEM, TZP, tobramycin (TOB). *S. aureus* was tested against penicillin (PEN), clindamycin (CLI), erythromycin (ERY), fusidic acid (FA), GEN, SXT and FOX and enterococci was tested against GEN, and vancomycin (VAN). Enterococci were also examined for high-level aminoglycoside resistance (HLAR) using GEN (30ug) where a zone diameter of ≤ 8mm was deemed as positive for HLAR. *S. aureus* isolates with FOX inhibition zones below 22mm were considered putative MRSA and shipped to Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res), University Hospital of North Norway, Tromsø, Norway, for molecular analyses by an in-house validated *mecA* and *nuc*-gene real-time PCR[12].

Enterobacterales species with reduced susceptibility to 3rd generation cephalosporins (CTX and/or CAZ), FOX, and/or MEM were further analysed for ESBL -, AmpC β-lactamase (AmpC) -, and carbapenemase-production, respectively, as described in EUCAST guidelines[13]. Briefly, the combination disc test was used to detect ESBL and AmpC using ROSCO kits (ROSCO, Taastrup, Denmark)[14]. ESBL production in *E. coli, Klebsiella* spp. and *P. mirabilis* was examined using the ROSCO ESBL confirm kit which contains CTX and CAZ tablets with or without the β-lactamase-inhibitor clavulanic acid. Cefepime with or without clavulanic acid was additionally tested for *Enterobacter* spp. Increased AmpC-production (AmpC-phenotype) was determined by the ROSCO AmpC Confirm ID kit (ROSCO), using CTX and CAZ tablets with and without the AmpC-inhibitor cloxacillin. Interpretation criteria of ESBL- and AmpC detection was ≥5mm increase in the growth inhibition zone diameter between the cephalosporin in combination with the

inhibitor compared to the cephalosporin alone. *P. aeruginosa* and *A. baumannii* isolates were not examined for ESBL and AmpC. A MEM (10ug) growth inhibition zone diameter of <27mm was used for screening of carbapenemase production in *Enterobacterales* using the ROSCO KPC/ metallo-β-lactamase and OXA-48 Confirm kit (ROSCO)[14]. Molecular verification of the ESBL- and AmpC phenotypes was not performed. They were thus presumed phenotypes.

The KPC-3 producing *K. pneumoniae* NCTC 13438, ESBL-positive K. pneumoniae NCTC 13368/ATCC 700603, S. aureus NCTC 12493- methicillin resistant (mecA), as well as wild-type *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used for quality control on a monthly basis and for each new batch of media or reagents.

Table 1. Numbers and proportions of clinical specimens with bacterial growth

Specimen type	Positive n/N (%)
Pus	255/403 (63)
Blood culture	25/153 (16)
Urine	31/88 (35)
Other body fluids	17/37 (46)
CSF	8/13 (62)
Total	336/694 (48)

Table 2. Distribution of ESKAPE pathogens, *E.coli, P.mirabilis* and *Salmonella spp.* by specimen type

Species	Pus	Blood	Urine	Other*	CSF	Total
Gram-negative	170/403 (42)	11/153 (7)	31/88 (35)	14/37(38)	3/13 (23)	229/327 (70)
Enterobacterales	128/403 (32)	8/153 (5)	26/88 (30)	10/37(27)	2/13 (15)	174/327 (53)
E. coli	65/403 (16)	3/153 (2)	18/88 (20)	5/37 (14)	1/13 (8)	92/327 (28)
E. cloacae	10 /403 (2)	1/153 (1)	1/88 (1)	1/37 (3)	-	13/327 (4)
K. pneumoniae	18/403 (5)	1/153 (1)	6/88 (7)	3/37 (8)	1/13 (8)	29/327 (9)
P. mirabilis	31/403 (8)	1/153 (1)	1/88 (1)	-	-	33/327 (10)
Salmonella spp.	4/403 (1)	2/153 (1)	-	1/37 (3)	-	7/327 (2)
Non-Enterobacterales	42/403 (10)	3/153 (2)	5/88 (6)	4/37 (11)	1/13 (8)	55/327 (17)
A. baumannii	18/403 (4)	3/153 (2)	2/88 (2)	3/37 (8)	-	26/327 (8)
P. aeruginosa	24/403 (6)	-	3/88 (3)	1/37 (3)	1/13 (8)	29/327 (9)
Gram- positive	85/403 (21)	4/153 (3)	4/88 (5)	5/37 (14)	-	98/327(30)
S. aureus	77/403 (19)	4/153 (3)	2/88 (2)	3/37 (8)	-	86/327(26)
Enterococcus	8/403 (2)	-	2/88 (2)	2/37 (5)	-	12/327 (4)

Data analysis

All data were entered into a Microsoft Excel 2010 © sheet and analysed using EpiInfoTM 7   software (CDC, USA). Data were described using percentages and frequencies.

Ethical Consideration

This study was approved by the Malawian College of Medicine Research and Ethics Committee; (reference no. P.11/17/2308) and by the University of KwaZulu Natal Biomedical Research Ethics Committee (reference no.BE093/16). Voluntary informed consent was sought from every patient prior to inclusion in the study.

Results

Overall culture results

A total of 694 specimens were collected from 367 males and 327 (47%) females aged between 18 – 89 years (mean 36 years). Each patient was entered only once in the study. More than one bacterial species was isolated from some samples. The specimens were received from the departments of surgery (n=370; 53%), medicine (n=230; 33%), obstetrics-gynaecology (n=87; 13%) and other wards (n=7; 1%). Specimens comprised of pus swabs (n=403; 58%), blood cultures (n= 153; 22%), urine (n=88; 13%), CSF (n=13; 2%), and other samples (n=37; 5%) which included ascites fluid (n=8), knee aspirate (n=8), pleural fluid (n=6), peritoneal fluid (n=7), sinus aspirate (n=5), middle ear aspirate (n=2), and a throat swab (n=1).

A total of 336/694 (48%) specimens yielded visible bacterial growth (Table 1): pus (255/403; 63%), urine (31/88; 35%), blood cultures (25/153; 16%), CSF (8/13; 62%), and other samples (17/37; 46%).   A total of 411 phenotypically different isolates were recovered from these 336 specimens. A total of 327 isolates (80%) were identified as ESKAPE pathogens (n=195/327; 60%), *E. coli* (n=92/327; 28%), *P. mirabilis* (n=33/327; 10%) or *Salmonella* spp. (n=7/327; 2%). The 327 isolates were recovered from 251 specimens and included for further analysis (Figure 1). Other bacterial species (n=84; 20%) were coagulase negative staphylococci (CNS) (n=25;6%) and non-ESKAPE pathogens (n=59;14%) including β-haemolytic streptococci (n=23), *Pseudomonas spp.* other than *P. aeruginosa* (n=2), *Kluyvera spp.* (n=1), *Moraxella spp.* (n=1), *Pantoea spp.* (n=3), *Providencia spp.* (n=1), *Raoultella spp.* (n=3), *Aeromonas spp.* (n=2), *Erwinia spp.* (n=1), *K.oxytoca* (n=2), *P.vulgaris* (n=1), *C. sedlakii* (n=4). *E. aerogenes* (n=1), and unidentified oxidase negative Gram-negative rods (n=14; 3%) were excluded from further analysis (Figure 1). The final study sample selected for AST consisted of Gram-negative bacilli (n=229; 70%) and Gram-positive cocci (n=98; 30%) (Figure 2).

Table 2 illustrates the distribution of ESKAPE pathogens, *E. coli, P. mirabilis* and *Salmonella spp.* by specimen type.

Bacterial species distribution

E. coli (n=92; 28%) and *S. aureus* (n=86; 26%) were the most commonly detected species. Pus, blood culture and urine samples yielded the highest number of pathogens (Table

Table 3. AST results for *Enterobacterales*, *P.aeruginosa* and *A.baumannii* given as numbers and percentages of isolates with reduced susceptibility (I + R category) to the individual antibiotics.

Antibiotics*	Enterobacterales						Non-Enterobacterales		
	Enterobacterales	E.coli	E. cloacae	K. pneumoniae	P. mirabilis	Salmonella spp.	Non-Enterobacterales	P. aeruginosa	A.baumannii
AMP	146/174 (84)	77/92 (84)	11/13 (85)	29/29 (100)	22/33 (67)	7/7 (100)	NT	NT*	NT
AZT	104/174 (60)	56/92 (61)	10/13 (77)	24/29 (83)	14/33 (42)	0/7 (0)	29/29 (100)	29/29 (100)	NT
CTX	106/174 (61)	56/92 (61)	9/13 (69)	24/29 (83)	17/33 (52)	0/7 (0)	NT	NT	NT
FOX	95/174 (55)	50/92 (54)	13/13 (100)	20/29 (69)	9/33 (27)	3/7 (43)	NT	NT	NT
CAZ	102 /174 (59)	53/92 (58)	8/13 (62)	24/29 (83)	17/33 (52)	0 /7 (0)	5/29 (17)	5/29 (17)	NT
CXM	112/174 (64)	58/92 (63)	8/13 (62)	24/29 (83)	22/33 (67)	0 /7 (0)	NT	NT	NT
CIP	94 /174 (54)	53/92 (58)	7/13 (54)	18/29 (62)	16 /33 (48)	0 /7 (0)	27/55 (49)	5/29 (17)	22/26 (85)
GEN	99 /174 (57)	52/92 (57)	7/13 (54)	24/29 (83)	15/33 (45)	1 /7 (14)	22/26 (85)	NT	22/26 (85)
MEM	8 /174 (5)	3/92 (3)	1/13 (8)	3/29 (10)	1 /33 (3)	0 /7 (0)	23/55 (42)	18/29 (66)#†	4/26 (15)
TZP	45/174 (26)	26/92 (28)	4/13 (31)	13/29 (45)	2/33 (6)	0/7 (0)	2/29 (7)	2/29 (7)	NT
SXT	140/174 (80)	79/92 (86)	9/13 (69)	25/29 (86)	21/33 (64)	6/7 (86)	26/26 (100)	NT	26/26 s(100)
TOB	NT	NT	NT	NT	NT	NT	2/29 (7)	2/29 (7)	NT

*Abbreviations: AMP=ampicillin, AZT= aztreonam, CTX=cefotaxime, FOX=cefoxitin, CAZ=ceftazidime, CXM=cefuroxime, CIP=ciprofloxacin, GEN=gentamicin, MEM=meropenem, TZP=piperacillin-tazobactam, SXT=trimethoprim-sulfamethoxazole, TOB=tobramycin, NT= not tested. # Including R-category (n=10/29; 34%) or I-category (n=8/29; 28%). †negative for carbapenemase production by molecular testing

Table 4. AST results for Gram-positive cocci given as number of isolates and the percentages with reduced susceptibility (I + R category) to the individual antibiotics

Antibiotics*	S.aureus	Enterococcus.
PEN	81/86 (94)	NT*
ERY	41/86 (48)	NT
CLI	20/86 (23)	NT
FA	7 /86 (8)	NT
VAN	NT	0 /12 (0)
GEN	21/86 (24)	0/12 (0)
SXT	27/86 (31)	NT
FOX	9/86 (10.5)	NT

Figure 2. Overall numbers and proportions of ESKAPE pathogens, *E.coli*, *P.mirabilis,* and *Salmonella spp.* (n=327) included for AST.
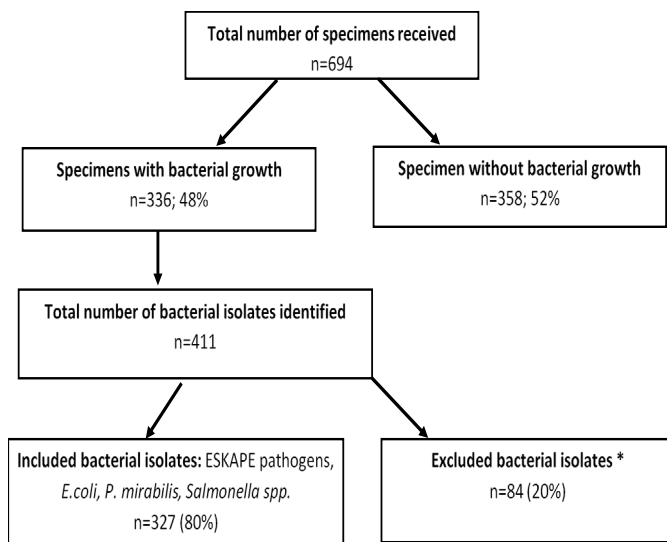

Figure 1. Flowchart of specimen analysis, bacterial growth and selection of clinical isolates for further analysis

*Coagulase negative staphylococci (CNS) (n=25;6%) and non-ESKAPE pathogens (n=59; 14%) including *B-haemolytic streptococci* (n=23), *Pseudomonas spp. other than P. aeruginosa* (n=2), *Kluyvera sp.* (n=1), *Moraxella* sp. (n=1), *Pantoea sp* (n=3), *Providencia sp.* (n=1), *Raoultella sp.* (n=3), *Aeromonas sp.* (n=2), *Erwinia sp.* (n=1), *K.oxytoca* (n=2), *P.vulgaris* (n=1), *C. sedlakii* (n=4). *E. aerogenes* (n=1), and unidentified oxidase negative Gram-negative rods (n=14; 3%).
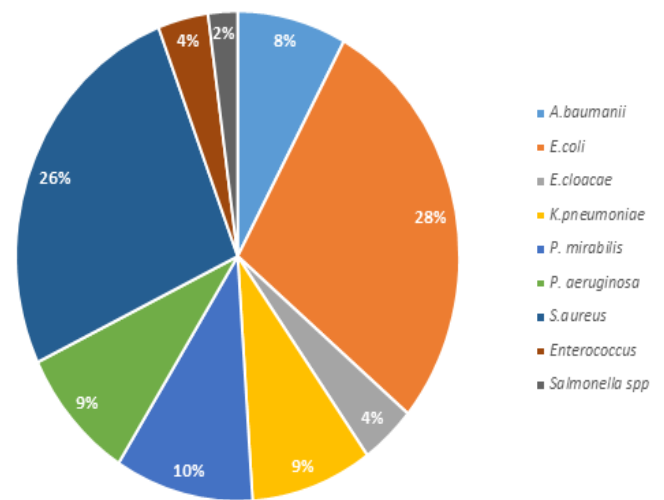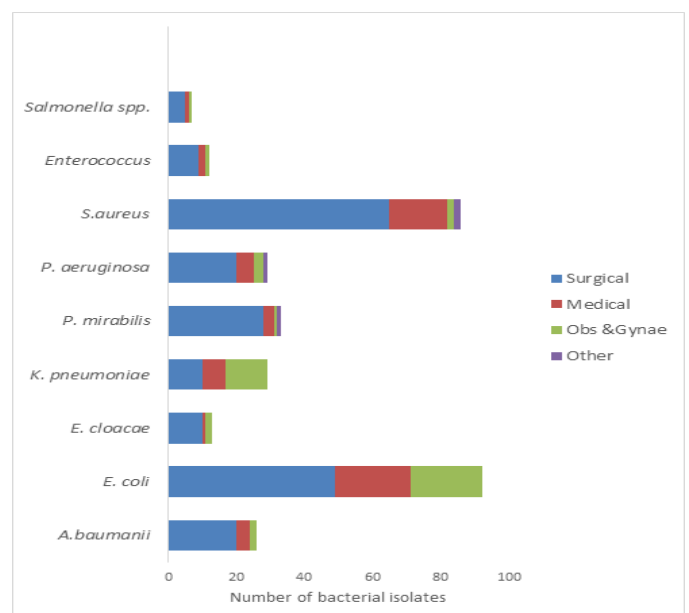

Figure 3. Distribution of ESKAPE pathogens, E.coli, P. mirabilis, and Salmonella spp. by hospital departments

2): (i) Gram-negative pathogens were recovered from pus (n=170/403; 42%), urine (n= 31/88; 35%), blood cultures (n=11/153; 7%), other body fluids (n=14/37; 35%) and CSF (n=3/13; 23%). (ii) Gram-positive pathogens were recovered from pus (85/403; 21%), urine (4/88; 5%), blood cultures (n= 4/153; 3%), and other body fluids (n=5/37; 14%) (Table 2).

Pathogens were recovered from several different departments (Figure 3). The surgical department dominated in overall numbers with *S. aureus* (n=65/86; 76%), and *E. coli* (n=49/92; 53%). The proportion of *E. coli* (n=22/92; 24%) and *S. aureus* (n=17/86; 20%) were also relatively high in specimens from the medical department. In the obstetrics and gynaecology department, *E. coli* (n=21/92; 23%) and *K. pneumoniae* (n=12/29; 41 %) were the most prevalent bacterial species.

Antimicrobial susceptibility and multidrug resistance

The overall AST-results for Gram-negative bacteria are presented in Table 3. We focused on the prevalence of reduced susceptibility to CTX and/or CAZ and associated co-resistance. *K. pneumoniae* (n=24/29; 83%), *E. cloacae* (n=9/13; 69%), *E. coli* (n=58/92; 63%), and *P. mirabilis* (n=17/33; 52%) showed reduced susceptibility to CTX and/ or CAZ, of which the majority were also MDR and resistant to CIP, GEN and SXT. MDR phenotypes were evident in *K. pneumoniae* (n=18/29; 62%), *E. cloacae* (n=5/13; 38%), *E. coli* (n=40/92; 43%), and *P. mirabilis* (n=13/33; 39%). All *Salmonella spp.* were susceptible to CTX and CAZ. ESBL-production was phenotypically confirmed in *K. pneumoniae* (n=20/29; 69%), *E. coli* (n=49/92; 53%), *E. cloacae* (n=6/13; 46%) and *P. mirabilis* (n=11/33; 33%). MDR was observed in a majority of the ESBL-positive isolates, i.e., *K. pneumoniae* (n=16/20; 80%), *E. cloacae* (n=4/6; 67%), *E. coli* (n=30/49; 61%) and *P. mirabilis* (n=8/11; 73%). In contrast, lower rates of MDR were observed in ESBL-negative isolates; *E. coli* (n=10/43; 23%), *E. cloacae* (n=2/5; 40%), *P. mirabilis* (n=5/22; 23%), *K. pneumoniae* (n=2/9; 22%).

An AmpC-phenotype was most prevalent in *E. cloacae* (n=5/13; 38%), followed by *P. mirabilis* (n=10/33; 30%), *K. pneumoniae* (n=8/29; 28%) and *E. coli* (n=26/92; 28%). The majority of isolates with AmpC phenotypes were also MDR, i.e., *E. coli* (n=10/26; 38%), *E. cloacae* (n=3/5; 60%), *K. pneumoniae* (n=6/8; 75%) and *P. mirabilis* (n=8/10; 80%). In comparison, lower rates of MDR were observed in AmpC negative isolates; *E. cloacae* (n=2/8; 25%), *K. pneumoniae* (n=12/21; 57%) and *P. mirabilis* (n=5/23; 22%) except for *E. coli* (n=30/66; 45%). A combined ESBL- and AmpC-phenotype was observed in all four *Enterobacterales*, i.e., *K. pneumoniae* (n=8/29; 28%), *E. coli* (n=20/92; 22%), *P. mirabilis* (n=6/33; 18%) and *E. cloacae* (3/13; 23%). A large proportion of these isolates were also MDR; *P. mirabilis* (n=5/6; 83%), *K. pneumoniae* (n=6/8; 75%), *E. cloacae* (n=2/3; 67%), *E. coli* (n=7/20; 35%).

The majority of *Enterobacterales E. coli* (66/92; 72%), *P. mirabilis* (31/33; 94%), *E. cloacae* (9/13; 69%) and *Salmonella* spp. (7/7; 100%) showed high levels of susceptibility to TZP except *K. pneumoniae* (n=16/29; 55%). For MEM, high levels of susceptibility were observed across all *Enterobacterales*; E. coli (89/92; 97%), *K. pneumoniae* (26/29; 90%), *P. mirabilis* (32/33; 97%), *E. cloacae* (12/13; 92%) and *Salmonella spp.* (7/7; 100%). *Enterobacterales* isolates with reduced susceptibility to MEM were examined by the ROSCO carbapenemase kit with

negative results (data not shown) indicating a combination of reduced permeability and ESBL-/AmpC-production rather than carbapenemases production as an explanation for reduced MEM susceptibility.

For *A. baumannii* and *P. aeruginosa* we focused primarily on reduced susceptibility to MEM. We observed a high rate of reduced susceptibility to MEM in *P. aeruginosa*, R-category (n=10/29; 34%) or I-category (n=8/29; 28%), with evenly distributed growth inhibition zone diameters between 6-23 mm, in contrast to *A. baumannii* (R-category n=3/26; 12% and I-category n=1/26; 4%). Carbapenemase production was not evident in MEM-R *P. aeruginosa* and *A. baumannii* using biochemical and molecular methods (K-res, Norway; data not shown). High levels of resistance was observed in *A. baumannii* against CIP (n=22/26; 85%) and GEN (n=22/26; 85%):

The overall AST results for *S. aureus* and *enterococci* are presented in Table 4. For *S. aureus* we focused on reduced susceptibility to FOX as a marker for presumptive MRSA and associated resistance. Although we observed a high initial rate of reduced susceptibility to FOX (38/86; 44%), MRSA was confirmed in only nine of the 38 isolates re-examined at K-res, resulting in an actual 10.5% MRSA prevalence (n=9/86). Almost all non-confirmed isolates had FOX inhibition zones of 19-21 mm, just below the screening breakpoint of 22 mm. The confirmed MRSA-isolates expressed reduced susceptibility to ERY (n=7/9; 78%) or GEN (n=8/9; 89%) or both ERY and GEN (n=7/9; 78%). In contrast, lower resistance rates were observed in MSSA isolates; ERY (n=33/77; 43%), GEN (n=12/77; 16%) and both GEN and ERY (n=11/77; 14%). For *Enterococcus* we focus on reduced susceptibility to VAN and high-level aminoglycoside resistance (HLAR), neither of which were observed (Table 4).

Discussion

We undertook a prospective observational study of antimicrobial susceptibility in clinically important bacterial pathogens obtained from adult in-patients at a governmental referral hospital for the central region of Malawi during a six months period in 2017. A similar study, also including children, was performed 10 years before our study at the same hospital and could provide comparative hospital-wide baseline data[6]. However, this study only included specimens collected at admission, whereas our study also included specimens during hospitalisation.

During the six-month study period, 22 524 unique admissions were registered in KCH, of which 16 237 were suspected to have a bacterial infection and were prescribed antibiotics. We obtained 694 specimens from unique patients which is relatively low in comparison to the overall number of patient with a potential bacterial infection. This is a typical observation in resource limited settings due to the lack of robust clinical microbiology services (CMS) and inadequate financial resources. However, the number of specimens collected during this study is similar to the number (n=2236) obtained during the 18 months study in 2006-7 when adjusted for study length[6]. This observation implies that most infections are still treated empirically without any microbiology investigations to determine the causative pathogen and its susceptibility profile[15-16]. This could be attributed to clinicians receiving little or no training in microbiology informed antibiotic prescribing, diagnostic stewardship, and availability of the antibiotics in

the hospital, similar to a recent Egyptian survey[16] finding that 63% of the physicians didn't receive any training on prescribing antibiotics and relied on their seniors or previous experience for prescribing antibiotics[16]. The Egyptian survey also showed that less than half of all antibiotics (44.5%) were prescribed following a microbiology result, 51% were prescribed on patient demand, and 68.2% based on availability of the antibiotics in hospital[16]. Moreover, it may also suggest that sampling is prioritised for the most critically ill patient and/or those not responding to empirical antibiotic treatment. This may create a bias in the overall sampling towards complicated infections and/or diagnostics in treatment failure.

The underuse of CMS may also in part be due to the lack of trust in and knowledge of the importance of local rates of antimicrobial resistance and consequences for therapy as shown in a recent Cambodian survey[17]. The Cambodian study revealed that the use of CMS was facilitated when results and microbiology staff were readily accessible, and if clinicians had trust and confidence in CMS while appreciating the importance of the results in clinical decision-making[17]. During our study we strengthened the interface between the laboratory and the clinicians by building capacity in terms of supplies and consumables, deploying focal contact persons at each ward and providing rapid laboratory results through a WhatsApp group. Caution was taken when using this platform to ensure confidentiality of patients by limiting WhatsApp groups to selected clinicians approved as department focal persons by heads of Department. We believe that these measures were important and allowed us to reach a number of specimens equivalent to the previous KCH study[6].

The overall proportion of specimens supporting bacterial growth was 48%, varying between the highest yield for pus samples (63%) and lowest for blood culture (16%). Low yield of blood cultures may have been attributed to blood cultures primarily collected from patient's already in hospital and on antibiotic therapy supressing bacterial growth. The limited yield of fastidious bacteria such as *S. pneumoniae* support the notion that the majority of blood cultures collected represented hospital infections and not community acquired infections[5]. The proportion of positive blood culture samples is consistent with the previous KCH-study[6]. The lack of visible bacterial growth in a third of pus samples could also be due to ongoing antibiotic treatment suppressing bacterial growth and/or the lack of anaerobic culturing, but the proportion of culture positive samples was comparable to that obtained in pus from burn wounds (74%) in hospitalised patients in Blantyre[18]. Unfortunately, the clinical information provided in the data collection form was limited and did not allow reliable information on the recent use of antibiotics nor any distinction of pus samples between primary abscesses, postoperative wound infections or other wound types.

E. coli and *S. aureus* were the most common species recovered across all specimen types representing 54% of the total isolates. The relative proportions were consistent with observations in the previous KCH-study from 2006-7 although their specimens were dominated by blood culture samples in contrast to our collection consisting of nearly 60% of pus samples[6]. The differences in the relative proportions of different specimen types precludes any further comparisons in bacterial species distributions

between the two studies performed at KCH. The relative dominance of *E. coli* and *S. aureus* in similar specimen types has been confirmed in other studies from Sub-Saharan African countries[5,18-22]. The differences in the prevalence of bacterial species between the different hospital departments could be partly explained by their corresponding dominant specimen type, e.g. the predominance of *E. coli* at the medical department from urine samples, and *S. aureus* in the surgical department recovered from pus specimens.

Importantly, we observed an overall high rate of MDR in the most common Gram-negative bacterial pathogens. Reduced susceptibility to most antimicrobials except MEM and TZP was observed in *Enterobacterales*. A high proportion (>50%) of ESBL-producing isolates was observed for all *Enterobacterales* species with reduced susceptibility to third generation cephalosporins except *Salmonella*. A substantial proportion of ESBL-producing isolates also revealed an AmpC-phenotype indicating the presence of plasmid-mediated AmpC, at least for *K. pneumoniae* and *P. mirabilis* species which do not harbour any intrinsic blaAmpC. ESBL- and AmpC phenotypes were categorised as presumed phenotypes as molecular verification was not performed. The clinical importance of co-resistance is illustrated by the high proportion of MDR in ESBL- and AmpC-producing isolates. The high rate of ESBL-producing *Enterobacterales* isolates is consistent with recent observations of MDR invasive isolates of *E. coli* and *K. pneumoniae* at the Queen Elizabeth Hospital in Blantyre[5], and seems to have increased significantly compared to the previous KCH-study[6]. In fact the current resistance pattern in *Enterobacterales* advises against the current use of the recommended first and second line antibiotics (PEN, CIP, GEN, ceftriaxone and amoxicillin-clavulanic acid) as prescribed in Malawi Standard Treatment Guidelines (MSTG) 5th Edition 2015 incorporating the Malawi Essential Medicine List (MEML) 2015[23]. It must be noted that although ceftriaxone was not directly a part of the antibiotic testing panel, representative marker antibiotics (CTX and CAZ) were tested. We did not examine *E. coli*, *Klebsiella spp.* and *P. mirabilis* for amoxicillin-clavulanic acid susceptibility, which still could be useful in the treatment of UTIs caused by ESBL-producing *Enterobacterales*. The antibiotic panels consisted of antibiotics that were available at the hospital pharmacy and actually being used on the ground and ensured that the panels of antibiotics that are tested are in line with international guidelines in particular for tracking ESBL and AmpC phenotypes. The data provided by this study may contribute to further development of national and local guidelines for antibiotic use also allowing antibiotic procurement planning.

The high proportion of resistance to extended-spectrum cephalosporins in clinical isolates of *Enterobacterales* has been reported from several sub-Saharan countries[5,24-26]. These antibiotics are commonly used and readily accessible over the counter without the need for a prescription in LMICs[4,27]. Overuse and misuse often leads to resistance and the findings of this study clearly indicate that the use and prescription of extended spectrum cephalosporins needs to be reviewed and closely monitored.

We observed a high-rate of MEM-resistance in *P. aeruginosa* compared to *A. baumannii*. Extended analyses of selected *P. aeruginosa* and *A. baumannii* isolates did not reveal any carbapenemases-producing isolates (data not shown). Thus, the reduced susceptibility to MEM may be attributed to

chromosomal mutational mechanisms affecting permeability, efflux mechanisms and hyper-production of AmpC[28]. The rate of confirmed MRSA do not indicate a significant increase compared to the previous KCH study [6]. Fortunately, we did not observe any VAN resistance nor HLAR in the few available clinical *enterococci*.

There are several limitations in this study including a relative short study period and limitations in sample size. The relatively low proportion of samples compared to the overall number of patients with a potential bacterial infection may indicate a sampling bias. Moreover, ESBL- and AmpC-phenotypes have not yet been confirmed by molecular methods and we have not done any phylogenetic typing to disclose clonal relatedness among isolates. On the other hand we have performed a prospective study and managed to mobilize the clinical departments to submit samples in a relative proportion that is comparable to the most recent study at KCH[6]. Moreover, bacterial culture (except anaerobic culturing) and AST has been performed according to international standards.

Conclusion

The overall results have documented a relatively high proportion of clinically important AMR-phenotypes consistent with ESBL-/AmpC-producing Enterobacterales, which strongly limits antimicrobial treatment options. The proportion of these phenotypes have increased since the last study at KCH in contrast to MRSA. These observations have consequences for empirical and targeted antimicrobial treatment as well as CMS and infection control at KCH. Urgent attention is required from local government, and in country public health committees with guidance from global health committees to address the problem. This could be achieved through establishment of local and national antibiotic stewardship programs, development of locally adapted clinical microbiology services, and AMR surveillance.

Declarations

Ethics approval and consent to participate

This study was approved by the Malawian College of Medicine Research and Ethics Committee; (reference no. P.11/17/2308) and by the University of KwaZulu Natal Biomedical Research Ethics Committee (reference no.BE093/16). Voluntary informed consent was sought from every patient prior to inclusion in the study.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed in this study are available from the corresponding author on reasonable request.

Competing interests

Sabiha Essack is chairperson of the Global Respiratory Infection Partnership and member of the Global Hygiene Council, both sponsored by an unrestricted educational grant from Reckitt UK. "The other authors declare that they have no competing interests"

Funding

Authors' contributions

FEC: Study design, sample processing, data collection, data analysis and interpretation, writing the manuscript

BCH: study design, data analysis, technical guidance, review of manuscript

IN: sample processing, data collection

OS: sample processing, data collection

TM: sample processing, data collection

FL: administrative guidance, review of manuscript

GSS: study design and review of manuscript

SYE: study design, review of manuscript

AS: study design, data analysis and interpretation, writing the manuscript

"All authors have read and approved the final manuscript"

Acknowledgements

References

1. O'neill J. Tackling drug-resistant infections globally: final report and recommendations. Wellcome Trust. 2016. https://amr-review.org/sites/default/files/160525_Final paper_with cover.pdf

2. World Health Organisation. Global Action Plan on Antimicrobial Resistance. Geneva: World Health Organisation. September 2015. http://www.asmscience.org/content/journal/microbe/10.1128/microbe.10.354.1

3. Tadesse BT, Ashley EA, Ongarello S, et al. Antimicrobial resistance in Africa: a systematic review. BMC Infcet. Dis. 2017; 17: 616. doi: 10.1186/s12879-017-2713-1

4. Leopold SJ, Van Leth F, Tarekegn H, Schultsz C. Antimicrobial drug resistance among clinically relevant bacterial isolates in sub-Saharan Africa: A systematic review. J Antimicrob Chemother. 2014; 69(9):2337–53. doi: 10.1093/jac/dku176

5. Musicha P, Cornick JE, Bar-Zeev N, et al. Trends in antimicrobial resistance in bloodstream infection isolates at a large urban hospital in Malawi (1998–2016): a surveillance study. Lancet Infect Dis. 2017;17(10):1042–52. doi: 10.1016/S1473-3099(17)30394-8

6. Makoka MH, Miller WC, Hoffman IF, et al. Bacterial infections in Lilongwe, Malawi: aetiology and antibiotic resistance. BMC Infect Dis. 2012; 12(1):67. doi: 10.1186/1471-2334-12-67

7. Gallaher JR, Banda W, Lachiewicz AM, Krysiak R, Cairns BA, Charles AG. Colonization with Multidrug-Resistant Enterobacteriaceae is Associated with Increased Mortality Following Burn Injury in Sub-Saharan Africa. World J Surg. 2018;42(10):3089–96. doi: 10.1007/s00268-018-4633-7

8. Karlowsky JA, Hoban DJ, Hackel MA, Lob SH, Sahm DF. Resistance among Gram-negative ESKAPE pathogens isolated from hospitalized patients with intra-abdominal and urinary tract infections in Latin American countries: SMART 2013–2015. Brazilian J Infect Dis. 2017;21(3):343–8. doi: 10.1016/j.bjid.2017.03.006

9. Government of Malawi. Malawi population and housing census report-2018. National Statistics Office 2019. http://www.nsomalawi.mw/images/stories/data_on_line/demography/census_2018/2018 Malawi Population and Housing Census Main Report.pdf

10. EUCAST. Antimicrobial susceptibility testing EUCAST disk diffusion method version 7.0. January 2019. https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Disk_test_documents/2019_manuals/Manual_v_7.0_EUCAST_Disk_Test_2019.pdf

11. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81. doi: 10.1111/j.1469-0691.2011.03570.x

12. Sundsfjord A, Simonsen GS, Haldorsen BC, et al. Genetic methods for detection of antimicrobial resistance. APMIS. 2004 Dec;112(11–12):815–37. doi: 10.1111/j.1600-0463.2004.apm11211-1208.x

13. Skov R, Skov G. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and / or epidemiological importance. 2012; 1–47.

14. Hansen F, Hammerum AM, Skov RL, Giske CG, Sundsfjord A, Samuelsen Ø. Evaluation of Rosco Neo-Sensitabs for phenotypic detection and subgrouping of ESBL-, AmpC- and carbapenemase-producing Enterobacteriaceae. APMIS. 2012;120(9):724–32. doi: 10.1111/j.1600-0463.2012.02898.x

15. Mandomando I, Sigaúque B, Morais L, et al. Antimicrobial drug resistance trends of bacteremia isolates in a rural hospital in southern Mozambique. Am J Trop Med Hyg. 2010;83(1):152–7. doi: 10.4269/ajtmh.2010.09-0578

16. Abdel Wahed WY, Ahmed EI, Hassan SK, Ibrahim EG, Eid HM. Physicians' knowledge, attitudes, and practice concerning antimicrobial resistance & prescribing: a survey in Fayoum Governorate, Egypt. J Public Heal. 2020;28(4):429–36. doi: 10.1007/s10389-019-01027-x

17. Om C, Vlieghe E, McLaughlin JC, Daily F, McLaws ML. Antibiotic prescribing practices: A national survey of Cambodian physicians. Am J Infect Control. 2016;44(10):1144–8. doi: 10.1016/j.ajic.2016.03.062

18. Livimbi OM, Komolafe IO. Epidemiology and bacterial colonization of burn injuries in Blantyre. Malawi Med J. 2007;19(1):25–7. doi: 10.4314/mmj.v19i1.10929

19. Mohammed A, Seid ME, Gebrecherkos T, Tiruneh M, Moges F. Bacterial Isolates and Their Antimicrobial Susceptibility Patterns of Wound Infections among Inpatients and Outpatients Attending the University of Gondar Referral Hospital, Northwest Ethiopia. Int J Microbiol. 2017:1–10. doi: 10.1155/2017/8953829

20. Seyoum Asres G, Hailu Legese M, Woldearegay GM. Prevalence of Multidrug Resistant Bacteria in Postoperative Wound Infections at Tikur Anbessa Specialized Hospital , Addis Ababa , Ethiopia. Arch Med. 2017;9(4:12):1–9. doi: 10.21767/1989-5216.1000233

21. Kajumbula H, Fujita AW, Mbabazi O, et al. Antimicrobial drug resistance in blood culture isolates at a tertiary hospital, Uganda. Emerg Infect Dis. 2018;24(1):174–5. doi: 10.3201/eid2401.171112

22. Uwaezuoke J, Nnodim J. Bacteriology of Different Wound Infections and Their Antimicrobial Susceptibility Patterns in Owerri. J Progress Res Biol. 2015;1(1):1–6.

23. Malawi Ministry of Health. Malawi Standard Treatment Guidelines (MSTG) 5 th Edition-incorporating Malawi essential medicine list (MEML) . 2015. https://extranet.who.int/ncdccs/Data/MWI_D1_Malawi-Standard-Treatment-Guidelines-Essential-Medicines-List-2015.pdf

24. Gray KJ, Wilson LK, Phiri A, Corkill JE, French N, Hart CA. Identification and characterization of ceftriaxone resistance and extended-spectrum β-lactamases in Malawian bacteraemic Enterobacteriaceae. J Antimicrob Chemother. 2006;57(4):661–5. doi: 10.1093/jac/dkl037

25. Estaleva CEL, Zimba TF, Sekyere JO, et al. High prevalence of multidrug resistant ESBL- and plasmid mediated AmpC-producing clinical isolates of Escherichia coli at Maputo Central Hospital, Mozambique. BMC Infect Dis. 2021;21(1):4–9. doi: 10.1186/s12879-020-05696-y

26. Toy T, Pak GD, Duc TP, et al. Multicountry Distribution and Characterization of Extended-spectrum β-Lactamase-associated Gram-negative Bacteria from Bloodstream Infections in Sub-Saharan Africa. Clin Infect Dis. 2019;69(Suppl 6):S449–58. doi: 10.1093/cid/ciz450

27. Kadavanu T, Mathai D. Antimicrobial Stewardship Program (ASP). Med Updat. 2012;22:3–7.

28. Annear D, Black J, Govender S. Multilocus sequence typing of carbapenem resistant Pseudomonas aeruginosa isolates from patients presenting at port elizabeth hospitals, South Africa. African J Infect Dis. 2017;11(2):68–74. doi: 10.21010/ajid.v11i2.9