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ARTICLES

Full Length Research Paper

Assessment of the prevalence of extended-spectrum β-lactamase producing Gram-negative bacilli at the Charles De Gaulle Paediatric University Hospital (CDG-PUH), Ouagadougou, Burkina Faso

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Bacterial resistance to antibiotics is a serious concern in developing countries due to precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. This study was aimed to assess the prevalence of strains producing extended-spectrum beta-lactamase in the Gram negative bacteria isolated in the laboratory. The study was conducted in the Charles De Gaulle Paediatric University Hospital (Ouagadougou) and the Arnaud de Villeneuve Regional University Hospital (Montpellier). Out of the 889 pathological substances (pus, urine and blood) analysed, 175 germs were isolated among which 110 were Gram negative bacteria (62.8%). 48.2% of the Gram negative bacteria were positive to extended-spectrum beta-lactamase. Among the 110 Gram negative bacteria isolated, 101 were Enterobacteria and 9 other Gram negative bacteria. No extended-spectrum beta-lactamase was found in the other Gram negative bacteria and 52.5% of Enterobacteria were extended-spectrum beta-lactamase positive. As for the extended-spectrum beta-lactamase phenotype, 60.4% were *Escherichia coli* and 32% were *Klebsiella pneumoniae*. 50.9% of extended-spectrum beta-lactamase came from pus and 41.5% from urine. In addition, 64.6% of hospitalised patients had an extended-spectrum beta-lactamase phenotype compared to 24.5% for out-patients. The results show the importance of the phenomenon and should help to better take care of this scourge because antibiotics despite everything, always save millions of lives.

Key words: Extended-spectrum beta-lactamase (ESBL), enterobacteria, gram-negative bacilli, inpatients, out-patients.

INTRODUCTION

As a global concern, bacterial resistance to antibiotics is   becoming a serious worry in developing countries due to

precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. Gram Negative Bacilli (GNB) are frequently implicated in human infections and Enterobacteria are the most commonly isolated bacterial species in the laboratory.

The secretion of extended-spectrum beta-lactamase (ESBL), a common mechanism of bacterial resistance to antibiotics, is becoming a threat to public health. Indeed, the ESBL bacteria are more and more resistant to all beta-lactams (except cephamycins and Carbapenems). In addition, the plasmids supports have bigger size and have genes resistant to other antibiotics causing Multidrug resistant (MDR) bacteria emergence (Boyd et al., 2004).

Perhaps in developed countries, the extended surveillance and the particular care provided to ESBL carriers have reduced the antimicrobial resistance; however, in under-developed countries, the unlimited accessibility to beta-lactams and the abusive use of those molecules contribute to expand the antibiotics resistance phenomenon (Livermore, 1995; Sangare et al., 2015; Storberg, 2014). In Burkina, this phenomenon is particularly marked in recent years with the free dispensing of ceftriaxone generic drug. From 15% in 2007 (Ouedraogo et al., 2011), the drug resistance went to 33% in 2013 (Ouedraogo et al., 2016).

The study aims to assess the scope of the phenomenon of bacterial resistance to antibiotics in the developing countries due to precarious hygiene conditions, inappropriate prescription as well as self-medication and free sale of antibiotics. The primary objective was to evaluate the extent of the phenomenon and identify ESBL-producing strains among the GNB isolated in the Charles-de-Gaulle Paediatric University Hospital (CDG-PUH) laboratory. Such study should help for emergency measures to fight it.

MATERIALS AND METHODS

The study was conducted at the Charles De Gaulle Paediatric University Hospital (CDG-PUH) in Ouagadougou, Burkina Faso and the Arnaud de Villeneuve Regional University Hospital (ADV-RUH) in Montpellier, France. It is a prospective cross-sectional descriptive study covering from 1st May to 12 August 2014.

The study population consisted of all patients who performed a bacteriological examination in the CDG-PUH laboratory. Included in this study were all patients in whom bacteriological analysis of the specimen (pus, urine and blood) resulted in isolation and identification of a GNB during the study period. Patients' or accompanying relatives' consent was received before the beginning of this research. Samples containing GNB were seeded on appropriate culture media for isolation. Non-selective media (CLED

agar) and / or selective (Hektöen agar, EMB) were used according to the pathological product and incubated in an oven at 37°C for 24 to 48 h.

The GNB obtained were reisolated on Muller Hinton medium to obtain pure cultures for preservation. The pure colonies were scraped with a seed and discharged into a cryotube containing a preservation medium (the "Protect" medium) and stored at -20°C until shipped to Montpellier in France, in Arnaud de Villeneuve Regional University Hospital (ADV-RUH). The Matrix-Assisted Laser Desorption/Ionisation-Time-of-Flight (MALDI-TOF) (automated bacteriology), the principle of which is based on mass spectrometry, was used to identify and analyze the bacterial protein. The antibiotic susceptibility testing was carried out through the dissemination technique on Mueller Hinton Agar plate according to antimicrobial committee of France microbiology society (CA-SFM) and the complete automated interpretation was done with SIRScan micro™.

Statistical analyses were performed using Epi-Info 7.2. Groups were compared using Pearson's or Yates χ² test, when needed. For all analyses, a p-value lower than 0.05 was considered as significant.

RESULTS

Socio-demographic characteristics of sample

In this study, 106 patients were included. Male patients were the majority (54.7%), giving a sex ratio of 1:2. The age group 1 to 30 months was the most important one. Out of the 106 patients, 60.4% were in-patients compared to 39.6% out-patients. Most of the hospitalised patients were from the surgical ward and represented 59.4%. Sixty six patients took antibiotics before the bacteriological test and represented 62.3% (Table 1).

Bacteriological results

Out of 889 pathological substances analysed, 175 germs have been isolated among which 110 (62.8%) were Gram negative bacteria. These 110 Gram negative bacteria were from 106 samples among which 102 were monomicrobials and 4 bimicrobials. A predominance of *E. coli* (51.8%) was observed, followed by *K. pneumoniae* with 26 germs representing 23.6%. *P. aeruginosa* came in third position with 7 germs representing 6.4%. Most of the microbes were found in urine and pus with a distribution of 55 cases (50%) and 46 cases (41.8%), respectively.

Out of the 110 germs isolated, 53 (48.2%) produced an extended spectrum of β-Lactamase. We also noted 101 enterobacteria and 9 other Gram negative bacteria. No production of extended spectrum of β-lactamase was

Table 1. Socio-demographic characteristics.

Age (months)	Number (n)	Percentage (%)
< 1	09	08
1-30	39	37
31-60	07	07
61-120	24	23
121-180	14	13
> 180	13	12
Total	106	100
Sex		
Male	58	55
Female	48	45
Total	106	100
Status		
In-patients	64	60
Out-patients	42	40
Total	106	100
Services		
Surgery	38	59
Older children	07	11
Infectious diseases	05	08
Neonatology	05	08
Infant	04	06
Others	05	08
Total	64	100

observed in the other Gram negative bacteria; the 53 ESBL producing germs were enterobacteria and represented 52.5%. As for the ESBL Phenotype, according to the species/gender, 60.4% of ESBL were *E coli* and 32% were *K pneumoniae,* 5.7% were *Enterobacter cloacae* and 1.9% were *Providencia stuartii* (Table 2). Most ESBL were found with *Escherichia coli* and *Klebsiella pneumonia* both representing 92.4% cases respectively equivalent to 60.4 and 32% of the cases.

In relation to each species, strong prevalence of ESBL has been observed: 65.4% *Klebsiella pneumoniae* had the ESBL phenotype; thereafter, *Escherichia coli* with 56.1% cases; *Enterobacter cloacae* and *Providencia stuartii* were in the third position with each 50% of cases (Table 2). The ESBL production frequency was 24.5% in out-patients and 64.6% in hospitalised ones. The value of $p < 0.05$ implies that there is a significant difference between the ESBL production within in-patients compared to out-patients. There are about 6 times (Odds Ratio = 5.64) more ESBL production risks in hospitalised patients than in non-resident ones (Table 3).

According to previous antibiotherapy, 79.2% ESBL production germs came from patients who had taken antibiotics in the last 3 months before the bacteriological test. ESBL producing bacteria were found in patients aged 1 to 30 months (32.1%) followed by patients aged 61 to 120 months (26.4 %) (Table 4).

Out of the 110 isolates, 43 (39.1%) manifested a resistance phenotype to β-lactams different from the ESBL phenotype. However, no carbapenemase phenotype has was observed (Figure 1).

Resistance phenotypes of isolates to aminoglycosides

Out of the 110 isolates, 60 (54.5%) presented at least one of the resistance phenotypes to aminoglycoside. *Klebsiella pneumoniae* manifested the highest resistance to aminoglycoside with 69.2% cases.

The GTNt phenotype (gentamicin, tobramycin, and netilmicin resistance) was mostly represented. No resistance phenotype to aminoglycoside was found in *Pseudomonas aeruginosa.*

Table 2. Distribution of ESBL producing germs according to bacterial species identified.

Identified specie	ESBL		
	Number (n)	Percentage (%) (related to ESBL total : n=53)	Percentage (%) (related to each species)
K. pneumoniae	17	32.0	65.4
E. coli	32	60.4	56.1
Enterobacter cloacae	3	5.7	50
Providencia stuartii	1	1.9	50
Total	53	100.0	-

Table 3. Distribution of ESBL producing germs according to status at hospital.

Status	ESBL		ESBL+		P
	N	Percentage (%)	N	Percentage (%)	
Out-patients	34	75.5	11	24.5	< 0.001
In-patients	23	35.4	42	64.6	

Yates' Chi square corrected = 15.6   p = 1.7 10^{-5}. Odds Ratio = 5.64.

Resistance of isolates to quinolones

Out of the 7 samples identified with *Pseudomonas aeruginosa*, 4 had a reduced sensitivity to quinolones and represented 57.1%. Among the isolates, 38.2% of them expressed resistance to all the quinolones. There was more resistance to nalidixic acid with 59.1% cases. *Escherichia coli* was the species that showed more resistance to all the quinolones tested and was followed by *Klebsiella pneumoniae*.

Cross resistance between different antibiotic families

Among the ESBL phenotype germs, 94.3% manifested a resistance phenotype to at least one quinolone and one aminoglycoside at the same time. 100% of ESBL producing germs manifested a resistance phenotype to at least 3 different families of antibiotics.

DISCUSSION

Of all the isolated enterobacteria, 52.5% were ESBL producers. This ESBL rate is above the 9% observed by Guessennd et al. (2008). This confirms the fact that ESBL producing strains are growing over the years (Guessennd et al., 2008). This proportion is close to that of Obeng-Nkrumah et al. (2013) who obtained 49.3% ESBL producing enterobacteria, and Métuor-Dabiré et al. (2014) who found 56 to 63.3%. This high quantity of ESBL producing bacteria in this study can be justified by

the very poor health and environmental hygiene conditions, the lack of a rigorous prescription and access to antibiotics in hospitals and drug stores, the street medicines phenomenon, the high cost of life pushing patients to stop treatment well before the deadline. This high ESBL production of this study species could also be explained by the fact that many of the microorganisms in this study have been isolated in samples of patients who came back for controls after antibiotic treatment. The isolated germs in these cases confirm the failure of the previous treatment and the germs become more resistant. Guessennd et al. (2008) in Côte d'Ivoire suspected the selection constraint used by practitioners and the presence of low concentrations of unmetabolised antibiotics released by the hospital to be a cause of MDR dissemination among which the ESBL producing Gram negative bacteria (Guessennd et al., 2008). A study on the hospitals discharge in Burkina Faso would permit to cast light on this possibility.

The ESBL producing germs according to the bacterial species

Most ESBL were found in *E. coli* and *K. pneumoniae,* both representing 92.4% cases equivalent to 60.4 and 32% cases respectively followed by *Enterobacter sp.* with 5.7% cases. The study results are similar to those of are among the commensal enterobacteria of the digestive tract, those that have a great capacity to become pathogenic. *E. coli* remains influential in the urinary tract infections; urine being the source of most of the samples

Table 4. ESBL distribution according to socio-demographic characteristics and nature of product.

Age (months)	ESBL	
	Number (n)	Percentage (%)
1-30	17	32.1
61-120	14	26.4
121-180	9	17.0
< 1	5	9.4
> 180	5	9.4
31-60	3	5.7
Total	53	100
Nature of sample		
Pus	27	50.9
Urine	22	41.5
Blood	4	7.6
Total	53	100
Services		
Surgery	24	57.1
Infant	4	9.5
Older children	4	9.5
Infectious diseases	3	7.2
Neonatology	3	7.2
Others	4	9.5
Total	42	100
Previous antibiotherapy		
Yes	42	79.2
Unknown	8	15.1
No	3	5.7
Total	53	100.0

received, that can explain why E. coli is the most isolated germ and in which more ESBL phenotype was found. *Klebsiella* is an enterobacteria very disseminated in hospitals and is associated to suppuration and to bacteremia (Obeng-Nkrumah et al., 2013).

Pus comes second after urine in the samples and justifies that *Klebsiella pneumoniae* is the second prevalent germ with the highest ESBL production. However, considering every bacterial species, we noticed there was more ESBL production in *Klebsiella pneumoniae* (65.4%), followed by *E. coli* (56.1%). This prevalence was also very prominent in *Enterobacter cloacae* and *Providencia stuartii with* 50% cases.

These figures are as high as those of Obeng-Nkrumah et al. (2013) who found that 75% *Enterobacter* cloacae, 61.5% *K. pneumoniae* and 43.7% *E.coli* showed the ESBL phenotype. Métuor-Dabiré et al. (2014) found superior figures equalling 69.38% for *Klebsiella* sp,

65.88% for *E.coli*, 75% for *Enterobacter* sp. and 58.33% for *Pseudomonas.* Also, Lonchel et al. (2012) in Cameroon in 2012 found 66.7% isolates of ESBL producing *E. coli*. This difference of proportions for *Pseudomonas* and the high proportion for ESBL producing *Providencia stuartii* might be explained by the low presence of these species in the bacterial population of our study. An important presence of these species would permit to better appreciate the ESBL production. The difference of proportion of ESBL producing *Enterobacter sp.* compared to the results of Métuor-Dabiré et al. (2014) and Obeng-Nkrumah et al. (2013) can also be justified by the lower quantity of this species in our bacterial population.

The highest ESBL prevalence in this study has been found with *K. pneumoniae.* We observed from many studies that the ESBL prevalence was higher for *K. pneumoniae* than for *E. coli* until the end of the years
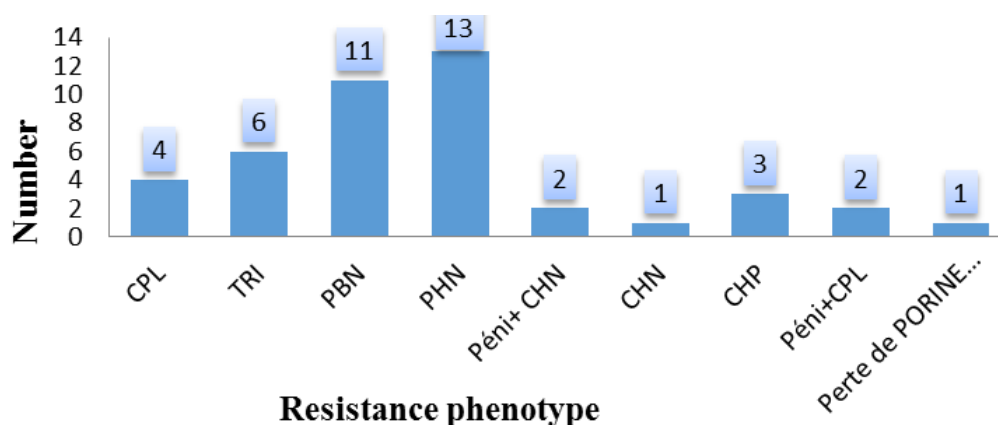
Figure 1. Distribution of the other resistance phenotypes to β-lactams.

1990s (Obeng-Nkrumah et al., 2013; Rodriguez-Villalobos and Struelens, 2006). But, nowadays, there is an immense change of the global epidemiological situation: ESBL producing strains are now mostly *E. coli* that is responsible for community infections and are mainly from urinary samples. This different result compared to our study could be due to the fact that most of our germs were isolated from hospitalised patients.

ESBL producing germs based on status at hospital

The frequency of ESBL production was 24.5% in out-patients against 64.6% in hospitalised ones. These figures are similar to those of Dabiré (2014) in Burkina Faso who reported 17% in out-patients against 83% in hospitalised ones. From these results we can deduce that hospitalisation is a risk factor for ESBL transmission. This observation has been made by Rodriguez-Villalobos and Struelens (2006) in Belgium that extended hospitalisation as a risk factor for ESBL producing germs dissemination.

ESBL producing germs based on the sample nature

100% germs from hemocultures showed ESBL phenotype followed by pus with 58.7% cases and urine with 40%. Ouédraogo et al. (2016) found that 62% urine and 57% pus contained ESBL producing germs. Obeng-Nkrumah et al. (2013) observed that 66.7% urine, 45.6% hemoculture samples and 0% pus contained ESBL producing germs. The primacy of hemoculture containing ESBL producing germs in this study might be explained by the low proportion of germs coming from these samples (3/110). There was an important proportion of ESBL producing germs in pus compared to urine; this result is different from those of Ouedraogo et al. (2011)

and Obeng-Nkrumah et al. (2013) who found an ESBL predominance in urine. Most of the pus samples in this study has been taken from hospitalized patients and 86.9% of those patients have taken antibiotics during their hospitalisation just before the sampling for bacteriological test. In addition, the third-generation cephalosporins (C3G) were the most used with Cefriaxone. Different studies show the impact of these antibiotics on the increase of ESBL enterobacteria production (Ouedraogo et al., 2011; Sangare et al., 2015). This phenomenon could explain the highest proportion of ESBL producing germs in pus compared to urine.

Other resistance phenotypes

Among the germs that do not have the ESBL phenotype, we found other resistance phenotypes to bêta-lactams whose impact on the bacterial resistance is not negligible. Indeed, 13 PHN phenotypes, 11 PBN, 6 TRI, 4 CPL, 3 CHP and 1 loss of Orp D2 porine have been recorded. This observation increases the phenomenon of resistance taking into account that the ESBL producing strains come from these penicillinases changes. No carbapenemase producing strains have been found. This is explained by the low availability of carbapenemase in our regions and its high cost; that is why it was found only in previous therapy of only 3 patients among the 106 entering the study which equals 2.8%. Among the germs having the ESBL phenotype, 94.3% manifested a resistance phenotype to at least one quinolone and one aminoglycoside at the same time; 100% of ESBL producing germs manifested a resistance phenotype to at least 3 different antibiotic families. These results confirm the observation by Boyd et al. (2004) who reported that ESBL are carried by large size plasmids which very often contain resistance genes to other antibiotic classes thus

making the host bacterium multi-resistant (Boyd et al., 2004).

Conclusion

From this study, it was observed that *E. coli* was the predominant species mainly found and about half of the isolates (48.2%) were ESBL+. In-patients had more risk to have an ESBL phenotype than out-patients. The high incidence of ESBL-secreting Gram negative bacteria should lead prescribers to improve the quality of their prescription, preferably based on the results of a correctly performed and interpreted antibiogram. The good delivery of antibiotics in officinal medicine and hygiene measures are points on which the focus must be put in order to reduce this global menace of bacterial resistance because despite the constant expansion of this phenomenon, it is also important to note that antibiotics still save millions of lives.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Incidence of bacterial infection in chronic hepatitis C virus (HCV) patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram- negative bacterial infections in the patients

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This study aimed to investigate the incidence of bacterial infection in chronic hepatitis C virus (HCV) patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram-negative bacterial infections in the patients. 100 HCV cirrhotic patients with ascites and 20 age- and sex-matched healthy subjects as control were included. Conventional culture methods were used to identify the causative organism of infection. Toll-like receptor 4 D299G polymorphism was detected by PCR- RFLP (polymerase chain reaction – restriction fragment length polymorphism). Patients were divided into: Group I: 100 HCV cirrhotic patients with ascites. They were subdivided into: Group I (a): Patients with Toll-like receptor 4 D299G polymorphism, Group I (b): Patients without polymorphism. Group II (control): 20 healthy subjects. This study showed significant higher incidence of infections in cirrhotic patients with Toll-like receptor 4 D299G polymorphism which play a role in the development of bacterial infection in cirrhotic patients that makes down-regulation of TLR4 response one of the immune mechanisms predisposed to Gram negative bacterial infection in cirrhotic patients.

Key words: TLR-4 gene polymorphism, cirrhosis, bacterial infection.

INTRODUCTION

Hepatitis C virus (HCV) is increasing health problems all over the world. It affects millions of people worldwide and is considered a leading cause of liver diseases including cirrhosis and hepatocellular carcinoma. Bacterial infections are considered the most frequent and severe complications in HCV patients with cirrhosis leading

to significant morbidity and mortality (Negro et al., 2014; Sadik et al., 2015). Development of bacterial infection in cirrhotic patients may be predisposed by change in the mechanism of antimicrobial defense (Mencin et al., 2009). It is known that the host genetic background can influence the outcome of HCV infection. Several polymorphisms were found to be associated with HCV infection. Mutations that alter the ability of innate immune receptors to bind their pathogen-associated molecular pattern (PAMPS) may also affect host susceptibility to infection (Schroder and Schumann, 2005). Toll-like receptors (TLRs) are a family of transmembrane receptors with extra cellular leucine-rich receptors and an intra-cellular signaling domain (Medvedev et al., 2013). TLR4, one of the most important and well-studied TLRs, is located on chromosome 9q32-33. TLR4 is known to recognize bacterial LPS but this receptor has also been found to recognize fusion protein from the respiratory syncytial virus (RSV) and the envelope protein of mouse mammary tumor virus (MMTV) (Bali et al., 2013). Reports have also shown that TLR4 can be stimulated by HCV nonstructural protein NS5A and thereby results in the secretion of IFNs and IL-6 from hepatocyte and B cells. The activation of TLR2 and TLR4 signaling in hepatocyte leads to upregulation of proinflammatory cytokines and chemokines, and recruitment of inflammatory cells to the liver (Bart-Ferwerda et al., 2008). In association of CD14 monocytes and its co-receptor MD-2 TLR4 triggers the inflammatory response to LPS of Gram negative bacteria and is a key factor in eliciting the systemic inflammatory response that can lead to sepsis, organ failure and septic shock (Mish and Hawn, 2008). The aim of this study is to investigate the incidence of bacterial infection in chronic HCV patients with cirrhosis and association between toll-like receptor 4 D299G gene polymorphism and Gram-negative bacterial infections in those patients.

METHODOLOGY

After approval of ethical committee in Tanta Faculty of Medicine and a written consent from all participants, this study was carried out on 100 HCV cirrhotic patients with ascites and 20 age- and sex-matched healthy subjects considered as control group. All patients were admitted to Tropical Medicine Department, Tanta University Hospital, Tanta, Egypt, during the period between Januaruy 2017 and January 2018. All patients and control were subjected to full history taking, clinical examination, routine laboratory investigations (liver function tests, complete blood picture, kidney function tests) and abdominal ultrasound for diagnosis of cirrhosis and real time – PCR for diagnosis of HCV.

Sampling

6 ml blood was taken under complete aseptic precautions and divided into two portions the first for routine assay and bacteriological study, and the other for the molecular study.

Inclusion criteria

HCV cirrhotic patients with ascites (child – Pugh C).

Exclusion criteria

(1) Patients taking immunomodulatory drugs.
(2) HCV coinfection with human immune deficiency virus or hepatitis B virus infection.
(3) Advanced hepatocellular carcinoma or extra hepatic malignancy.
(4) Recent history of previous infection (within previous 6 weeks).
(5) Hepatic encephalopathy in the previous 6 weeks.
(6) Treatment with antibiotics in the previous 6 weeks.
(7) Gastrointestinal bleeding in previous 7 days.

The patients were divided into:

Group I: 100 HCV cirrhotic patients with ascites that were subdivided according to presence of Toll-like receptor 4 D299G polymorphism into:

Group I (a) : Patients with Toll-like receptor 4 D299G polymorphism.
Group I (b) : Patients without polymorphism.

Group II: 20 healthy subjects as control.

PCR- RFLP (polymerase chain reaction – restriction fragment length polymorphism) was done to determine the presence of the Toll-like receptor 4 D299G polymorphism

Bacteriological study

Conventional culture techniques were used to identify the causative organism of bacterial infection which was confirmed by biochemical reactions.

Genomic DNA extraction and polymorphism genotyping

8 ml of peripheral blood was collected in EDTA tube and centrifuged at 3500 g for 10 min. Genomic DNA was extracted from buffy coat fraction using QIA mp DNA blood minikit (Qiagen Inc. Valencia, CA, USA) . The primer sequences used for TLR-4 Asp299Gly genotyping were 5-GATTAGCATACTTAGACTACCTCCATG-3 and 5-GATCAACTTCTGAAAAAGCATTCCCAC-3. The polymerase chain reaction (PCR) consisted of an initial denaturation at 95 C, 30 s at 57 C and 1 min at 72 C. Once the amplification was confirmed, the PCR product was digested for 1 h at 37 C with restriction enzyme NcoI. The change of A to G at position 896 produced a site for the restriction enzyme NcoI. The digested fragments of PCR amplification were analyzed by electrophoresis on 2.5 % agarose gel (Zhang et al, 2013)

Statistical analysis

Statistical package for social sciences (SPSS) package (version 9.0) was used for data analysis.

RESULTS

There was no statistical difference between cirrhotic patients and control as regard age and gender ($P > 0.05$). Table 1 showed statistically significant difference

Table 1. Clinical and laboratory data in all groups.

Variable	Cirrhosis before AB (n=100)	Cirrhosis after AB (n=100)	Control (n=20)	P
Age	53.57±4.12	54.83±9.31	49.37±10.68	0.753
Gender (m/f)	58/42	58/42	11/9	1.000
Bilirubin (mg/dl)	1.8±0.4	1.6±1.0	0.8±0.5	<0.001*
ALT (IU/L)	59.5±47.3	66.4±22.8	24.0±15.2	<0.001*
AST (IU/L)	70.8±63.0	83.5±35.7	33.2±14.6	<0.001*
Albumin (g/dl)	3.2±0.7	3.1±0.5	4.8±0.3	<0.001*
PT (seconds)	14.5±2.8	13.7±0.8	11.6±0.4	<0.001*
Platelet(10^3/mm^3)	157.7±87.5	105.4±24.5	241.3±34.8	<0.001*
Hb (gm/dl)	9±0.5	9.5±0.7	12±1.3	< 0.001*
WBC(cell/cmm)	3.6±1.1	2.7±0.71	4.5±1.4	<0.001*
S.creatinine (mg/dl)	2.21±0.15	1.52±0.22	o.73±0.19	<0.001*

All data are expressed in terms of mean ± standard deviation. $P< 0.05$ *significant. ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; PT, Prothrombin time; AB, Antibiotic.

Table 2. Type of infections in cirrhotic patients with and without polymorphism.

Variable	Polymorphism (n=10) N (%)	No polymorphism (n=90) N (%)	P
SBP	5 50	10 11.1	0.030*
Gram -ve organism	4 40	4 4.4	0.029*
Gram +ve organism	1 10	6 6.7	0.029*
UTI	4 40	7 7.8	0.049*
Gram -ve organism	4 40	5 5.5	1.000
Gram +ve organism	0 00.00	2 2.3	1.000
Chest infections	3 30	3 3.3	0.023*
Ellulitis	3 30	2 2.2	0.006*
Encephalopathy	6 60	15 16.7	0.037*
Bacteremia	4 40	4 4.4	0.004*
Gram -ve organism	3 30	2 2.2	0.945
Gram +ve organism	1 10	2 2.2	0.945

$P <0.05$ * Significant.

between cirrhotic and control as regard serum billirubin, ALT, AST, albumin, platelets count, prothrombin time, heamoglobin level white blood cells and creatinine. Serum creatinine was significantly higher in cirrhotic before antibiotic than after antibiotic ($p < 0.001$).

Table 2 revealed that 10 out of 100 (10%) cirrhotic patients are presented with Toll-like receptor 4 D299G polymorphism. Spontaneous bacterial peritonitis (SBP) infection was significantly higher in TLR-4 D299G polymorphism patients than those without polymorphism (50% Vs 11.1%) ($P < 0.05$). SBP caused by Gram negative bacteria in 40% of patients with polymorphism compare to 4.4% of those without. This was statistically significant ($P < 0.05$). Urinary tract infection was significantly higher in 40% of polymorphism group compare to 7.8% of patients without polymorphism $P<0.05$. The infection was mainly caused by Gram negative bacteria in both groups. Encephalopathy was found in 60% of patients with TLR-4 D299G polymorphism compare to 16.7% of patients without polymorphism. This was statistically significant ($P < 0.05$). The incidence of other types of infection as chest infection, cellulitis and bacteremia were significantly higher in polymorphism patients ($P < 0.05$).

Table 3. Microbilogical profile of infection of the studied group.

Site of infection	Peritonitis (%)	UTI (%)	Chest (%)	Cellulitis (%)	Encephalitis (%)	Bacteraemia (%)
Klebsiella	2 (2)	1 (1)	1 (1)	1 (1)	6 (6)	2 (2)
E.coli	3 (3)	3 (3)	1 (1)	1 (1)	5 (5)	0 (0)
Staph.aureus	5 (5)	2 (2)	1 (1)	0 (0)	2 (2)	3 (3)
Citrobacter	3 (3)	4 (4)	1 (1)	2 (2)	3 (3)	2 (2)
Enterococci	2(3)	1 (1)	2 (2)	1 (1)	5 (5)	1 ()

Table 4. Correlation between the TLR-4 polymorphosm and bacterial infection in cirrhotic patients.

Variable	R	P
TLR-4 gene polymorphism / infection	0.565	0.019*

P <0.05 * Significant.

Table 3 shows bacteriological profile of infection of the studied group. Table 4 positive correlation was found between TLR-4 gene polymorphism and incidence of infection in cirrhotic patients (r = 0.565, P = 0.019).

DISCUSSION

It has long been considered that immune response is impaired in patients with cirrhosis and that this predisposes bacterial infection. TLRs provide the most important early critical response to invading organism (Jover et al., 2009). TLR variant genotypes are associated with significantly increased serum levels of their specific antigenic ligands (LTA, LPS and bacterial DNA, respectively).

The results of this study showed significant higher incidence of bacterial infection in cirrhotic patients with ascites and TLR4 gene polymorphism than in cirrhotic patient without polymorphism. These findings came in accordance with the results reported by Argente et al. (2010) who observed a significant trend toward a higher incidence of bacterial infection and a significantly higher number of infections per patient in cirrhotic group withTLR4 D299G polymorphism.

In this study, TLR4D299G polymorphism was detected in 10% of cirrhotic patients with ascites. This result was nearly similar to the result of Argente et al. (2010), they reported incidence of about 9% of TLR4 D299G polymorphism in Child –Pugh C cirrhotic patients. Also, we revealed high incidence of encephalopathy in TLR4 D299G polymorphism group compared to cirrhotic group without polymorphism. This also was in accordance with the study of Argente et al. (2010), they reported higher incidence of encephalopathy in TLR-4 D299G polymorphism patients than in patients without polymorphism. They observed that this complication showed a trend toward a higher frequency during the study period in polymorphism group.

There are several explanation for high incidence of encephalopathy in these type of patients. Polymorphism itself could be a consequence of the high number of infections in these patients (Fernandez et al., 2007). This could also account for the higher serum creatinine levels in patients with TLR-4 D299G polymorphism, as they presented a tendency towards a higher incidence of infections as the cause of cirrhotic hospitalization (Jover et al., 2009). It could therefore be that a different inflammatory response related to the presence of the TLR-4 D299G polymorphism favors the development of encephalopathy as one of the most important complication (Frances et al., 2008). The results of this study showed a significant positive correlation between TLR4 gene polymorphism and incidence of infections in cirrhotic patients. These findings were also reported by Argente et al. (2010); they reported a significant correlation between TLR4 gene polymorphism and incidence of encephalopathy in cirrhotic patients. In other diseases, this significant positive correlation was observed by Yin et al. (2010), they reported TLR4 gene polymorphism is associated with increased risk of urinary tract infection in adults especially with acute cystitis and urethritis. This study showed significant impairment of TLR4 expression in PBMCs of cirrhotic patients in this study. This was in agreement with Tazi et al. (2006) and Testro et al. (2010), they revealed significant down regulation of TLR4 expression in PBMCs of cirrhotic patients. In contrast to this study finding, Stadlbauer et al. (2008) and Riordan et al. (2003) reported increased expression of TLR4 in monocytic cells of cirrhotic patients which showed return to normal level after antibiotic use. This disparity may be associated with the different etiology of the liver cirrhosis and/or different severe degrees of cirrhosis. A significant inverse correlation was detected between TLR4 expression and incidence of infection. These findings were similar to those reported

by Testro et al. (2010). All previous findings raised the possibility that, the high rate of Gram negative infections in patients with decompensated cirrhosis is not purely due to translocation of enteric organisms , but is contributed to an impairment of the TLR-4 dependent innate immune response. One potential explanation for these effects is that in response to repeated exposure to high concentration of Gram negative bacterial products and endotoxin, the TLR-4 dependent innate immune response is down regulated in an attempt to prevent chronic unopposed inflammation. In contrast to healthy control people, endotoxemia in cirrhotic patients does not lead to the typical systemic reaction, suggesting altered immune response to circulatory endotoxin in these patients (Manigold et al., 2003)

Conclusion

Toll-like receptor 4 D299G polymorphism has a role in the development of bacterial infection in cirrhotic patients that makes down-regulation of TLR4 response as one of the immune mechanisms predispose to Gram negative bacterial infection in cirrhotic patients.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Identification of insect and disease associated to citrus in Northern Ethiopia

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The survey was carried out in 2016 in the production of citrus fruits at Adiha and Sheka Tekli irrigation schemes of Kolla Tembien and Tanqua Abergelle districts, to assess the type of diseases, the causes and the characteristic symptoms of the diseases and to identify the types of insect pests that inflict serious damage. The laboratory analysis result depicted that bacteria and fungi caused most of the diseases of citrus at Adiha and Sheka Tekli irrigation schemes. Exceptionally cyst nematode and climbing weed were problems in production of citrus at the irrigation schemes. Diseases like citrus melanose, brown rot, root rot, blue mold, sooty mold, leaf spot and fruit spot were among the fungal diseases identified at the irrigation schemes. Moreover, citrus greening, and citrus canker were also some of the bacterial diseases of citrus at Adiha and Sheka Tekli irrigation schemes. Conversely, the type of insect pest that predominantly constrained the production of citrus in both areas were woolly whitefly, citrus mealybugs, orange dogs, cottony cushion scale insects, brown scale insect, citrus leafminer, citrus psyllid, fruit fly, adult flatid planthoppers, citrus aphid, red scale and root weevil respectively. Most of these insect pests belong to the Hemipteran order followed by lepidopteron insect group. Milk vine had also been observed as one of the weeds that pose serious problems to some of the citrus trees at Adiha and Sheka Tekli irrigation schemes. Therefore, further research intervention should focus on management of these insect and disease pests at Adiha and Sheka Tekli irrigation schemes.

Key words: Citrus disease, insect pest, symptoms and identification.

INTRODUCTION

Crop intensification is one of the strategies to increase productivity per unit area of land. Small-scale irrigation has been chosen as a strategic intervention to address food security in Ethiopia. This is because irrigation increases the potential for producing more food consistently in the drought-prone areas. Horticultural crops play a significant role in a developing country like Ethiopia, both in improving income and social spheres for the fulfilment of human nutrition. In addition, they help in maintaining ecological balance since they are so diverse. Furthermore, the sector provides employment opportunities for their management being too intensive to

labour and the sector is encouraging where countries having abundant labour and scarce capital like Ethiopia.

Citrus (*Citrus* spp.) constitutes the major group of fruits including oranges, grapefruits, trifoliate orange, mandarins, pummelo, citranges and lemon. It serves as the main source of vitamins, minerals elements and sugar; hence, it controls the building process of human bodies (Oviasogie et al., 2015). The crop has been produced widely in the tropical and subtropical regions of the world, in over 137 countries and generates around 105 billion US dollar per year (FAO, 2016). Based on FAO report, China, United States, Brazil, Italy and Mexico are the main producers of citrus that represent two–thirds of the global production. In Africa, Egypt, South Africa, Morocco, Algeria and Tunisia are the leading producers of Citrus. Ethiopia has a huge potential in the production of many types of fruits including citrus. Despite the exact time of citrus introduction to Ethiopia is not known, the start of its cultivation dates back to the early of 14 centuries by the monasteries of Gund Gundo (Northern Ethiopia). Later it has been cultivated in upper Awash valley and Melkassa in central Ethiopia. Currently, it is widely cultivated around Dire Dawa, lower and middle Awash and Melkassa areas in the southeast region of Ethiopia (Mekonen et al., 2015). According to the report of Kedebe (2015), about 61,472.74 ha of land were cultivated with a different type of fruits annually. Among the fruits, citrus is the leading fruit crops grown by many small-scale and commercial farmers (CSA, 2012). Hence, the annual production and productivity of citrus have estimated 5,947 ha and 77,087 tons, respectively (Dagnew et al., 2014). Based on the report of Mekonen et al. (2015), citrus occupied about 7290 ha of land in 1985, but later the coverage has reduced to 5380 ha with a production of 33500 metric tons.

Most parts of Tigray region have suitable agroecology for production of citrus. Recently the regional government has given prior attention for the expansion of small-scale irrigation to increase the production of vegetables and fruit crop. Thus, the regional Bureau of Agriculture and rural development and other Non-Governmental Organizations have introduced planting materials from across the corners of the country to different parts of the region including Kola Tembien and Tanqua Abergelle districts for the last few years. According to the Ethiopian Central Statistics Agency (CSA, 2012) report, the regional (Tigray) productivity and area coverage of citrus in 2001/2002 cropping season was not more than 1930 quintals and 48.2 ha respectively. However, the latest report indicated that the total area coverage and annual production of citrus in the region have been increased to 5,947 ha and 77,087 tons, respectively (CSA, 2013). The area under citrus cultivation in the region has increased by 99% within the last eight years. Among the fruits, citrus is widely cultivated crop both at Adiha and Sheka Tekli irrigation schemes. Over 163.75 ha of land has been planted with citrus at Adiha and Sheka Tekli irrigation

schemes. Despite its increase in acreage and production of citrus, the productivity still remains low (15 to 30 ton ha^{-1}) compared to the productivity of citrus in Brazil and USA (50 to 100 tone ha^{-1}) (FAO, 2014).

The decline in productivity of citrus has been attributed to several biotic and abiotic factors. Among the factors, many types of fungal, bacterial, viral, nematode, insect pests and less likely binding weeds seriously threaten the production and productivity of citrus. In Ethiopia, many bacterial and fungal diseases; mainly citrus canker, citrus greening, gummosis, anthracnose, Phaeoramularia leaf and fruit spot, melanose, blue and green mold of citrus fruits were recorded in many citrus farms of Awara Melka, Melka Werer, Merti Jeju, Aleta Wendo, Dale and Bebeka (Derso and Sijam, 2007; Yesuf, 2013). The specific cause of citrus greening named *Candidatus* Liberibacter Africanus subsp. clausenae and the vector (citrus psyllid) of the disease were identified in Ethiopia in 2016. Hence, the presence of the disease and the natural vector can lead to rapid spread of the diseases to many parts of Ethiopia (Agricultural Research Council-Plant Health Protection (ARC-PHP), 2017). Based on the reports of Mekbib et al. (2006), fruit pests inflict up to 80% of yield reduction in citrus fruits around Arbaminch. The yield loss of citrus varies among pests, as yield reduction due to *Pseudocercospora angolensis,* for instance*,* ranges from 50-100% (Yesuf, 2013). Another disease like gummosis also causes a yield loss of 10 to 30% throughout the world. The damage of gummosis increased with the use of susceptible rootstocks and application of excessive irrigation water (Al-Sadi et al., 2014). Among the citrus, sweet orange is susceptible to postharvest diseases, in which, the extent of damage range from 25.5 to 43.8% (Oviasogie et al., 2015). Similarly, citrus nematode cause damage to over 50 species of citrus that belongs to the Rutaceae family. Based on the reports of Irshad et al. (2012), nematode can cause up to 43.3% of yield loss in citrus.

The type of insect pests like Red scale, leaf miner, Orange dog, Mediterranean fruit fly, woolly whitefly, false codling moth, thrips, aphids and Bud mites have been identified so far as the main pests of citrus in many fruit growing parts of Ethiopia (Yosef et al., 2014). Among the insects, woolly whiteflies are the newly introduced alien invasive pests of citrus across many parts of Ethiopia. The pest sucks the saps of phloem, causing the leaf to wilt and drop when the population of the insect starts increasing. The droplets of the insects collect dust and provide a favourable environment for the growth of sooty mold (Getu, 2007).

Disease and insect pests are the main constraints to production of citrus trees at Adiha and Sheka Tekli irrigation schemes. Knowledge of the type of disease and insect pests is essential for appropriate monitoring leading to a devising of effective management strategies. However, the available information on the type of diseases, insect pests and weeds pose serious threats to
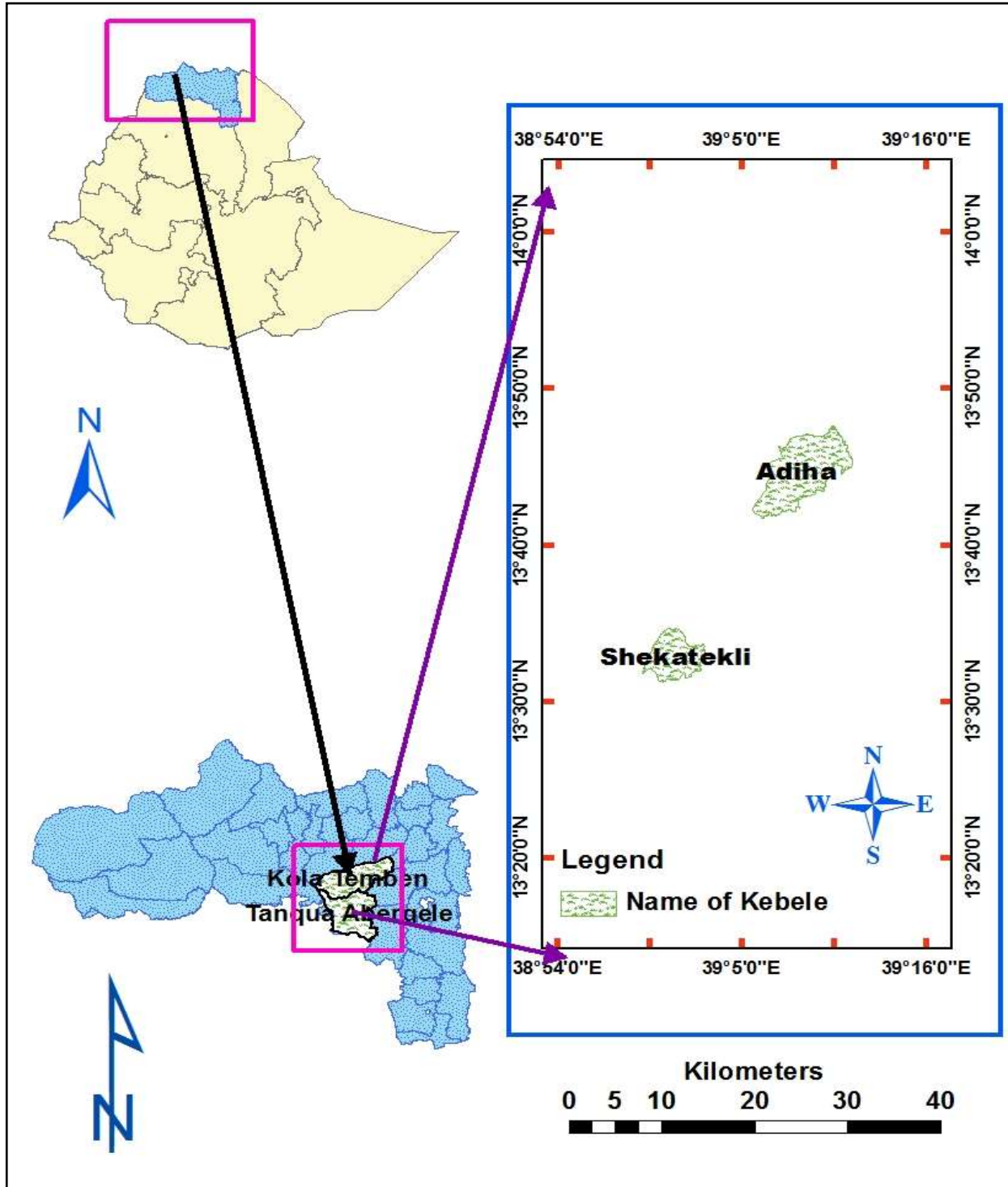
Figure 1. Map showing location of study sites.

production of citrus in the irrigation schemes as very limited. Therefore, field assessment was carried out to identify the main type of diseases, the causes and the characteristic symptoms of the diseases and to identify the types of insect pests that inflict serious damage in the irrigation schemes.

MATERIALS AND METHODS

Description of the study sites

The survey was carried out in Kola-Tembien and Tanqua Abergelle districts at Adiha and Sheka Tekli irrigation schemes (Figure 1). The study sites are located 35 km and 36 km away from Abyi-Addi and

Yechila administrative unit of Kolla Tembien and Tanqua Abergelle districts respectively. Adiha irrigation scheme is situated at an altitude of 1600-1750 m.a.s.l., whereas, Sheka Tekli irrigation scheme it is located at an elevation below 1500 m.a.s.l and the soil texture that dominantly comprises 58% sand soil and 42% silt loam soil types in both irrigation schemes. The mean annual rainfall and temperature range from 500-800 mm and 25 - 30°C respectively (Data of the Woreda's OoARD, 1998). Citrus is the main fruit that is widely grown in both locations since they have favourable environmental conditions and the presence of a year-round source of irrigation water.

Survey and sampling techniques

The survey was conducted in May 2016 at Adiha and Sheka Tekli irrigation schemes where citrus is widely planted in irrigation fields. Systematic sampling technique was employed to determine sampling fields. Hence, 10 orange-planted fields were assessed from each location. Thereafter, the diseased and insect-infested specimen was collected from the leaf, fruit and shoot part of the standing plant. Most insect pests were determined visually during the execution of the survey. Whereas those diseased plant specimens (including fruits, leaves and roots) that could not easily be distinguished on the field were collected, tagged and sent to plant protection laboratory in Ambo.

Sampling and handling technique

Plant parts with clear disease symptoms were selected from each sampled citrus tree. The specimens were collected using sterilized scatter and finally packed in transparent plastic bags. Eventually, each plastic bag was enveloped in brown paper bags and necessary information about the specimen written on it. Finally, specimens were placed in the icebox to prevent desiccation from sun and air currents and were thus sent to Ambo Plant Protection Center for further isolation and identification of the type and causal agents of the diseases.

Isolation and Identification of fungal pathogen

Diseased leaf and fruit samples were cut into larger pieces followed by washing with distilled water. Finally, they were surface sterilized using 70% ethanol followed by 5% Clorox for a minute and rinsed in distilled water three times. Sterilized leaves or fruit peels were cut into four discs or pieces and placed in Petri dishes containing potato dextrose agar (PDA) in five replicates and incubated at 25±1°C. Cultures were purified using hyphal tipping onto fresh PDA medium and were incubated for four to seven days at 25±1°C. The fungal isolates were identified by analyzing cultural and morphological features through examination of the growth pattern of colonies, morphological structure of conidia, development of fungal growth structures, pigmentation of the colonies, shape of the hyphal growth and mycelia (Thiyam and Sharma, 2013). The identification was carried out by placing a small portion of the aerial Mycelia from each culture on a clean slide with the help of mounting needle. The mycelium was properly spread on the slide using a needle. A coverslip was carefully placed and pressed gently over the spread to prevent the formation of air bubbles. Finally, the slide was then mounted and examined with help of a microscope. The morphological characteristics and features of the fungal organisms were examined and identified in accordance with Onuorah (2015).

Isolation, purification and identification of bacterial pathogens

Bacterial isolation, purification and identification were the first steps for plant bacteriological studies. The isolation of bacterial pathogens was done to obtain pure bacterial cultures, in which it was essential in the identification of the pathogens through analyzing the morphology, physiology and biochemical characteristics of a particular bacterial strain. The isolation of pure bacterial culture was carried out by taking pieces of leaf and fruit from sampled specimens. The pieces were washed and surface sterilized with distilled water, 70% alcohol and sodium hypochlorite respectively. Thereafter, the pieces were chopped into pieces using mortar and pistol until bacterial agents adequately released into the suspension. Finally, pure cultures were found by taking a loop of suspension and streak into general and differential media. The differential media was used for specific suspected bacterial pathogens. Eventually, the cultures were incubated for four to five days within the incubator under the specific requirement. The cultures were examined starting from 20 h of incubation for any signs of bacterial growth, color and shape of the colonies. Finally, a piece of culture was taken and examined using a binocular microscope. Therefore, the identification was accomplished by performing the morphological, physiological and biochemical tests and the results were compared to established identification schemes.

Nematode extraction and identification

A soil sample was taken from 30 cm of rooting depth of citrus and finally, one kilogram of soil sample together with some roots citrus was sampled and immediately sent to Ambo Plant Protection Research Center before soil loss its moisture. Eventually, 200 ml of soil was taken from the sample to extract nematode using Baermann technique. Nematodes were extracted after 24 h on aperture stainless steel sieve. Species identification was conducted after the transfer of female nematodes into anhydrous glycerol on permanent slide mounts following the method described by Hooper et al. (2005). Finally, the specimens were examined using compound microscope equipped with differential interference contrast at 630-1000x magnification. Finally, the presence and identification of the type of nematode was made following the image of scanned picture using DC 180 camera.

RESULTS AND DISCUSSION

Main diseases of citrus fruits at Adiha and Sheka Tekli irrigation scheme

Citrus greening

Citrus greening is an obligate bacterial disease that was serious in many citrus plantings at Adiha and Sheka Tekli irrigation schemes. Based on the biochemical characterization of the disease, the cause of the disease has been recognized as *Candidatus africanum.* The African citrus psyllid (*Trioza ertreae*) is common minor insect pest and transmits huanglongbing, also called citrus greening while feeding on the saps of the phloem. The psyllid was also another pest of citrus at Adiha and Sheka Tekli irrigation schemes. Hence, the disease could be transmitted from infected trees to a healthy one with the help of the vector so-called citrus Psyllid. According to the report of Agricultural Research Council-Plant Health Protection (ARC-PHP, 2017), the specific cause of the disease and its vector (citrus psyllid) were identified in

Figure 2. The image of citrus attacked by fungal, bacterial and vine weed at Adiha and Sheka Tekli irrigation scheme.

Ethiopia in 2016. Hence, the presence of the disease and the natural vector may lead to rapid spread of the diseases to many parts of Ethiopia including Adiha and Sheka Tekli citrus orchards. Citrus leaves infected with citrus greening showed a characteristic symptom of blotchy mottled and yellowing discolorations (Figure 2). In addition, the leaves were found to be narrowly structured and they did have a bunchy appearance. Severely infested citrus trees at Adiha and Sheka Tekli irrigation schemes ultimately remained as leafless twigs and dieback.

Citrus canker

The laboratory analysis result indicated that citrus canker was caused by *Xanthomonas citri* pv. citri. The disease resulted in premature leaf and fruit drop, twig dieback, eventual decline, and blemished citrus at both Adiha and Sheka Tekli irrigations schemes. The symptom of the disease was actually starting as tiny blister-like lesions and finally, it appeared as distinct necrotic lesions with a raised corky appearance that often had a yellow halo.

The diseased portion appeared scabby or corky. Citrus infested with canker had got dark brown to black raised lesions. Based on the survey conducted in 2008, the disease was recorded in many parts of the rift valley located at an altitude below 1300 m and it was confirmed to affect many citrus species across the rift valley of Ethiopia (Derso, 2009). He also mentions that several strains of canker have been proven to have wide host range of citrus. He confirmed that the Ethiopian strain of canker has had wide geographical distribution across many citrus growing African Regions. The planting materials of citrus had been introduced to Adiha and Sheka Tekli kebeles from major citrus growing parts of Ethiopia mostly from Gunda Gundo, lower and upper awash citrus farms. Therefore, the diseases may be introduced to these irrigation schemes associated with the planting materials.

Citrus melanose

Melanose disease affected young leaves and fruits of certain citrus at Adiha and Sheka Tekli irrigation schemes.

When rain and humid weather extend to certain period, the tissues expand and show variegated symptoms like small spots or scab-like lesions to patterns of damage referred to as teardrop, mud cake, and star melanose (Figure 2). That was one of the common diseases of citrus fruits at Adiha and Sheka Tekli farms. Examination of the spores of the fungus indicated that *Diaporthe citri* is the cause of the disease. It can create severe fruit rind blemishes, but the fungus does not normally affect the pulp. On leaves, the small, black, raised lesions are often surrounded by yellow halos and can cause leaf distortion. Based on the reports of Scot (2008), the disease spreads to nearby healthy citrus fruits through rain or overhead irrigation splash water. He also mentioned the ascospores of the fungus mainly dispersed to distant citrus fruits by wind currents. However, in all cases, the infection is caused by the conidia of the fungus. Similarly, the disease was recorded in many citrus farms of Ethiopia including Awara Melka, Melka Werer, Merti Jeju, Aleta Wendo, Dale, and Bebeka (Derso and Sijam, 2007).

Gummosis

The disease is caused by *Phytophthora citrophthora* and that was a well-known gumming disease of citrus at Adiha and Sheka Tekli irrigations schemes. It was characterized based on the formation of Gum on the trunk or branches. Tree with gummosis formed longitudinal cracking of bark, accompanied by brown gumming from the lesions. While the affected barks were removed from the base of the trunk, water soaked, reddish-brown, or in late stages black slimy appearance was revealed. Diseased bark would remove easily in recently affected citrus woods. Later, the trees collapse and die due to the girdling of the bark by the pathogen. Based on the report of Al-Sadi et al. (2014), severe infestation and the damage of gummosis to citrus occurred while farmers use susceptible rootstocks and during application of excessive irrigation water. Besides additional factors like freeze damage, high water table and salt accumulation also contribute to the development of the disease. Gummosis develops rapidly when moist, cool conditions prevail at both Adiha and Sheka Tekli irrigation schemes. However, there was seen slow spread and development of the diseases when hot summer weather appeared. Such conditions play an essential role in healing and drying of mechanical wounds.

Nematode

The root and soil sample examined in plant protection laboratory indicated that some numbers of citrus grown at Adiha and Sheka Tekli irrigation schemes were infested with nematode. The type of nematode identified in both schemes was known as *Tylenchulus semipenetrans* and

it was widely observed as parasitic nematode of citrus at both Adiha and Sheka Tekli irrigation schemes. The report of Irshad et al. (2012) indicated that citrus nematode inflicts damage to over 50 species of citrus across the world. He also added that the yield loss of citrus due to nematode was estimated to be 43.3% on average. According to the reports of Inserra et al. (2003), most citrus species are preferred host of nematode.

Sooty mold

Sooty mold damage was often observed in most plantations of Adiha and Sheka Tekli citrus fruits. It appeared as black discolouration on portions of the fruit and leaves following the massive infestation of woolly whiteflies. The sooty mold was a black thin mat of fungal growth usually observed on the upper leaf surfaces and fruit. It was grown following the excretion of sugary dew (honeydew) of sap sacking whiteflies, scale insects, aphids, psyllids, and mealy bugs. The current result is in line with the finding of Getu (2007), who stated that the droplets of some sap-sucking insects inflict the collection of specks of dust, which in turn provides an optimum condition for the growth of sooty mold. When the fungus becomes abundant, the mold could reduce photosynthesis and delay fruit colouring. Sooty mold represents a dramatic sign that insect population has reached damaging levels.

Blue mold

Blue mold was caused by *Penicillium italicum* Wehmer (most important on citrus). It was widely observed as postharvest diseases of citrus fruits at Adiha and Sheka Tekli irrigation schemes (Table 1). Initially, it appeared as a soft, water socked and slightly discolored spot and later enlarges in diameter within a few days when the daytime temperature increases. White mycelium then appeared on the surface of the fruit, and when the fungi grow blue colored spores were produced. Within a few days after the appearance of the diseases, the entire fruit surface was covered with blue colored spores. The spores do spread easily while fruits expose to wind. The laboratory analysis result was in accord with the findings of Oviasogie et al. (2015), who stated that blue molds were common airborne diseases that occur during post-harvest handling or storage of citrus fruits. The infestation of the diseases started at field and proceeds during packaging.

Main insect pests of citrus at Adiha and Sheka Tekli irrigation schemes

The result of the survey indicated that a considerable number of insect pest's constraint the production and productivity of citrus at Adiha and Sheka Tekli irrigation schemes. Most of these insects belong to the order

Table 1. Diseases that attack orange at Adiha and Sheka Tekli Irrigation scheme.

Common name	Order	Family	Causative agent
Citrus Greening	Rhizobiales	Rhizobiaceae	*Candidatus africanum*
Citrus Canker	Xanthomonadales	Xanthomonadaceae	*Xanthomonas citri* pv. citri
Orange Scab	Myriangiales	Elsinoaceae	*Elsinoe fawcettii*
Blue mold	Eurotiales	Trichocomaceae	*Penicillium italicum*
Gummosis	Pythiales	Pythiaceae	*Phytophthora citrophthora*
Sooty mold	Capnodiales	Davidiellaceae	*Cladosporium herbarium*
Black mold	Eurotiales	Trichocomaceae	*Aspergillus niger*
Nematode	Tylenchida	Tylenchulidae	*Tylenchulus semipenetrans*

Table 2. List of insect pests that attack orange at Adiha and Sheka Tekli irrigation scheme.

Common name	Order	Family	Causative agent
Wooly whitefly	Hemiptera	Aleyrodidae	*Aleurothrixus floccosus*
Citrus Mealybugs	Hemiptera	Pseudococcidae	*Planococcus citri*
Orange dogs	lepidoptera	Papilionidae	*Papilio demodocus*
Cottony cushion scale	Hemiptera	Margarodidae	*Icerya purchasi*
Brown scale	Hemiptera	Coccidae	*Coccus hesperidum*(L.)
Citrus Leaf minor	Lepidoptera	Gracillariidae	*Phyllocnistis citrella*
Citrus psyllid	Homoptera	Psyllidae	*Trioza erytreae*
Fruit fly	Diptera	Tephritidae	*Bacterocera spp*
Adult flatid planthoppers	Hemiptera	Flatidae	*Metcalfa pruinosa*
Citrus Aphid	Hemiptera	Aphididae	Toxoptera citricida
Root weevil	Coleoptera	Curculionidae	*Diaprepes abbreviatus*

Hemiptera followed by the lepidopteran insect pests. As depicted in the Table 2, woolly whitefly, citrus Mealybugs, Orange dogs, Cottony cushion scale, Brown scale insect, Citrus psyllid, Fruit fly, Adult flatid planthoppers, Citrus Aphid and Root weevil had been identified as the main pests of citrus at Adiha and Sheka Tekli irrigation schemes. Based on the reports of Yosef et al. (2014), many insect pests mainly Red scale insect, leaf miner, Orange dog, Mediterranean fruit fly, woolly whitefly, false codling moth, thrips, aphids and Bud mites were serious constraints of citrus across many parts of the nation. Insect pests like woolly whiteflies are the newly introduced alien invasive pests of citrus across many parts of Ethiopia. The pest sucks the saps of phloem, causing the leaf to wilt and drop when the population of the insect increases (Getu, 2007). Some insects like Adult flatid planthoppers and Root weevil were first recognized as pests of citrus at Adiha and Sheka Tekli irrigation schemes (Figure 3).

Relative occurrence of insect pests on citrus fruits

The relative occurrence of insect pests in citrus was classified according to the order of each insect that it belongs to. The result of the survey indicated that the Hemipteran insect pests were observed most frequently in many citrus plants of Adiha and Sheka Tekli irrigation schemes (Figure 4). The lepidopteran insect pests were also another threat to the production of citrus at Adiha and Sheka Tekli irrigation schemes following Hemipteran. However, the Homopteran, Dipteran and Coleopteran insect pests were less prevalent compared to the Hemipteran and lepidopteran insects.

Weed infestation

Apart from the insect and disease pests, there had been milkweed vine that had seriously threatened citrus trees at Adiha and Sheka Tekli irrigation schemes. Milkweed vine was the only weed that competes with citrus for water, nutrient, space and light in the irrigation schemes. In citrus orchards, the vine climbs the citrus trees, strangling tightly the stems and branches of the plant. The stem of the vine is woody, hard, inflexible and over tightening the collar and braches of citrus at Adiha and Sheka Tekli irrigation schemes (Table 3). The weed first originated in South America (Futch, 2006), but currently it has been observed in many citrus framings of Ethiopia

Figure 3. The image of citrus attacked by major insect pests at Adiha and Sheka Tekli irrigation scheme.
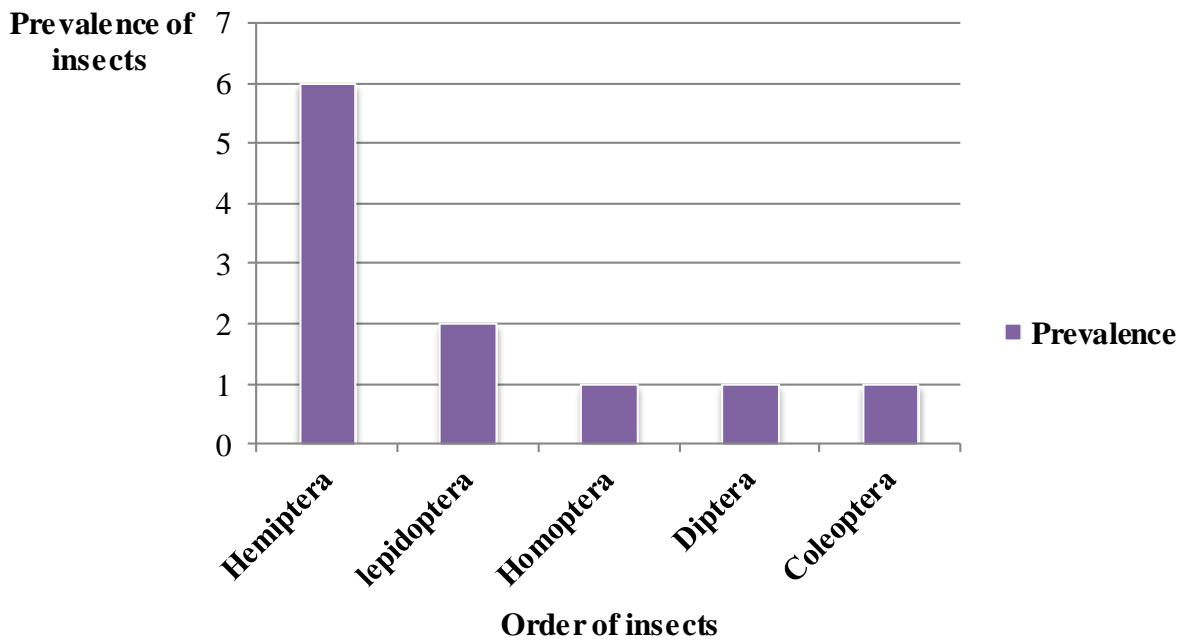

Figure 4. Graphical illustration of insect pests on citrus.

Table 3. Type of non-parasitic weeds of orange at Adiha and Sheka Tekli.

Common name	Order	Family	Scientific name
Milkweed vine (weed)	Rhamnales	Vitaceae	*Cissus trifoliata*

including the citrus farms at Adiha and Sheka Tekli irrigation schemes. According to Futch (2006), milkweed vines are broadleaf weeds with sail or parachute like structures on their seeds which help them to relocate to new locations.

Conclusion and recommendation

The production of citrus fruit is declining in the district. The occurrence of insect and disease pests of citrus leads to yield and quality reduction of orange. Ten most widely observed microbial pathogens of citrus have been identified at Adiha and Sheka Tekli irrigation schemes. Based on the result of the experiment, the main type of fungi and bacterial diseases that constraint the production and productivity of Citrus at Adiha and Sheka Tekli citrus farms were Citrus Greening, Citrus Canker, Orange Scab, Leaf and fruit Spot, Blue mold, Phytophthora foot rot, Nematode, Sooty mold, Brown rot and Black respectively. Besides, it has been found that 11 insect pests keep threatening the production of citrus at Adiha and Sheka Tekli citrus farms. Insects that belong to Hemiptera order followed by lepidopterans were predominant pests across the irrigation schemes. Therefore, further research intervention should focus on determination of the severity and abundance of each type of disease and insect pests of citrus at the irrigation schemes. Furthermore, any intervention should also gear towards management of those pests to improve the productivity of citrus at the irrigation schemes.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Assessment of antimicrobial resistance patterns in *Escherichia coli* isolated from clinical samples in Madinah, Saudi Arabia

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Escherichia coli **is a Gram-negative bacteria that causes various diseases, including pneumonia, urinary tract infections, and diarrhoea. The present work is an effort to study antimicrobial resistance pattern in this bacteria. Clinical samples (6840) were collected from King Fahd Hospital in Madinah, Saudi Arabia and screened for *E. coli* strains. Of all positive samples obtained from different clinical sources, about 3% isolates represented *E. coli* and 71.6% of these were collected from clinical samples of male patients. *E. coli* isolates were recovered from sputum (20.58%), wound (55.88%), and catheters tips (9.31%) representing about 86% of all clinical specimens. Antimicrobial susceptibility pattern of the *E. coli* isolates to twelve different antimicrobials revealed that all the isolates (100%) were susceptible to imipenem, amikacin, and aztreonam. Cefoxitin, ceftazidime and ciprofloxacin showed a sensitivity of 98.7%. This was followed by gentamycin (98.6%), piperacillin (95.7%), cotrimoxazole (92.3%), cephalothin (91.4%), and Augmentin (91.1%). Ampicillin showed the least susceptibility of 87.5%. Hence, cotrimoxazole, Augmentin, and ampicillin should be prescribed with care in order to avoid increasing resistance patterns in *E. coli*. Results also demonstrate that frequency of *E. coli* infections was highest during summer and winter seasons representing about 30% each. Autumn season, which coincided with the season of pilgrimage, recorded about 22% of infections while spring season had the least recorded percentage with only 17% of infections. This study is therefore a step towards the generation of national data on the prevalence of antimicrobial resistance patterns of *E. coli*.**

Key words: *Escherichia coli,* antimicrobial susceptibility pattern, multi-drug-resistance, antimicrobials.

INTRODUCTION

Multidrug resistant (MDR) has become a public health issue which is estimated to cause maximum deaths by the year 2050 along with increasingly high health expenses. Besides, not many effective drugs are available for the treatment of multidrug-resistant Gram-negative bacteria (Prestinaci et al., 2015; Alawi and Darvesh, 2016). Recent reports from Middle East including Saudi Arabia, show a considerable and

increasing prevalence of antimicrobial-resistant bacteria (Alawi and Darvesh, 2016; Zowawi, 2016). It has also been reported that although many physicians are aware of the dangers of MDR, majority of them do not comply with antimicrobial prescribing guidelines (Baadani et al., 2015; Al-Harthi et al., 2015).

Gram-negative bacteria, specifically those belonging to the family Enterobacteriaceae, can acquire genes that encode for multiple antimicrobial resistance mechanisms, including extended-spectrum-lactamases (ESBLs), AmpC-β-lactamase, and carbapenemases (Bader et al., 2017). One member of this group, *Escherichia coli* (*E. coli*) is ubiquitous and is present in both animals and the environment (Guenther et al., 2011). This gram-negative, facultatively anaerobic, rod-shaped, coliform bacteria is also the most common cause of food and water-borne human diarrhea worldwide, causing many deaths especially in young children (Hunter et al., 2003). It is the leading cause of urinary tract infections (UTIs), blood stream infections, wounds infections, otitis media and other complications in humans (Prestinaci et al., 2015). More than 80% of UTIs occur in outpatients and *E. coli* accounts for more than 50% of the infections in these patients (Kirac et al., 2016). A rise in antimicrobial resistance has been reported in *E. coli* worldwide which is causing complications and treatment issues (Zowawi, 2016). A number of studies have been done in Kingdom of Saudi Arabia (KSA) on the antimicrobial resistance patterns of *E. coli* from various clinical sources (Halawani, 2011; Masoud et al., 2011; Zowawi, 2016). The present study is another effort to determine antimicrobial susceptibility of *E. coli* from clinical sources at a busy hospital at Madinah, KSA.

MATERIALS AND METHODS

Sample collection

Different clinical samples such as sputum, wound swab, bile, tracheal aspirate (Tr. asp.), throat aspirate (Th. asp.), catheter tip, pus, abdominal abscess (Abd. ab.), ear swab, peritoneal wound swab (Peri. w.s.), pleural fluid (Pler. fluid), vaginal swab (VS), urethral discharge (UD), eye cornea swab (ECS), bone tissues, brain tube were collected from 6840 patients suspected of bacterial infection at King Fahd Hospital at Madinah, KSA. Clinical samples were cultured to isolate the organisms. Demographic data such as sex of the patients was recorded prior to sample collection.

Culture and identification

The clinical samples were collected and aseptically inoculated on blood agar, chocolate agar, cystine-lactose-electrolyte-deficient (CLED) agar and MacConkey agar (Oxoid Cambridge, UK) according to Centers for Disease Control and Prevention Guidelines (CDCP, 2013). The culture plates were incubated at 37°C for 24 h. Identification was done based on morphological characteristics of the colonies including size, shape, colour, pigmentation and haemolytic nature.

Biochemical characterization

Suspected *Escherichia coli* colonies isolated were further identified through biochemical tests (Barrow and Felthan, 2003) using standard procedures and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md). Identification included the following tests: Nitrate reduction test, citrate utilization test, oxidase test, H_2S gas production, methyl-red test, indole test, urease test, Voges-Proskauer test and lactose fermentation (Forbes et al., 2007).

Antimicrobial susceptibility test

Susceptibility to antimicrobial agents was determined by using the disk diffusion method (Oqunshe, 2006), and Phoenix automated microbiology 100 ID/AST system (Becton Dickinson Company, Sparks, Md.). The following antimicrobial agents obtained from BDH (London, UK) were used: Ampicillin (10 µg), Augmentin [amoxycillin + clavulinic acid (20/10 µg)], gentamycin (10 µg), cefoxitin (30 µg), cephalothin (30 µg), cotrimoxazole[trimethoprim-sulfamethoxazole 1:19 (25 µg)], amikacin (30 µg), ceftazidime (30 µg), aztreonam (30 µg), piperacillin (100 µg), imipenem (10 µg), and ciprofloxacin (5 µg). The inocula were prepared by growing the *E. coli* strains on separate agar plates and colonies from the plates were transferred with a loop into 3 ml of normal saline. The density of these suspensions was adjusted to 0.5 McFarland standards. The surface of Muller-Hinton agar (Oxoid Cambridge, UK) plate was evenly inoculated with the organisms using a sterile swab. The swab was dipped into the suspension and pressed against the side of the test tube to remove excess fluid. The wet swab was then used to inoculate the Muller-Hinton agar by evenly streaking across the surface. By means of a disc dispenser (Oxoid Cambridge, UK), the antimicrobial discs were applied onto the surface of the inoculated agar and the plates were incubated overnight at 37°C. The diameter of zone of growth inhibition observed was measured and compared to the chart provided by Clinical and Laboratory Standards Institute (CLSI, 2015).

RESULTS AND DISCUSSION

MDR is an alarming issue that is increasing continuously day by day; the main reason being inappropriate use and abuse of antimicrobials. Self-medication leads to patients consuming inadequate drug doses. MDR has to be monitored at several levels starting from basic research on how resistance develops in bacteria, to formulating strategies on regulating the dosage and susceptibility to different antimicrobials. When *E. coli* becomes resistant to carbapenems, like other bacteria of the Carbapenem-Resistant *Enterobacteriacae* (CRE) group, it becomes

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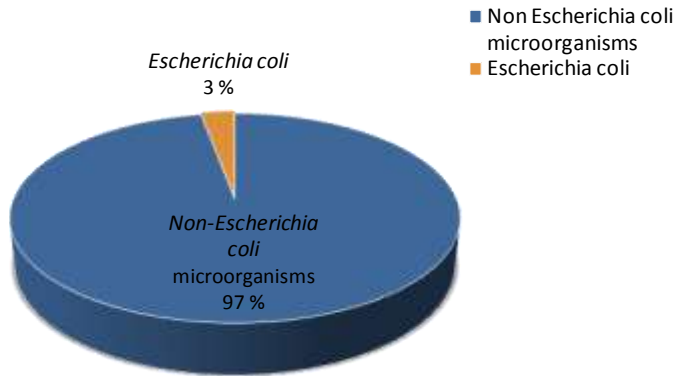#This author contributed equally: nikhatmanzoor@yahoo.co.in.

Figure 1. Percentage of *E. coli* specimens in comparison with other clinical isolates**.**
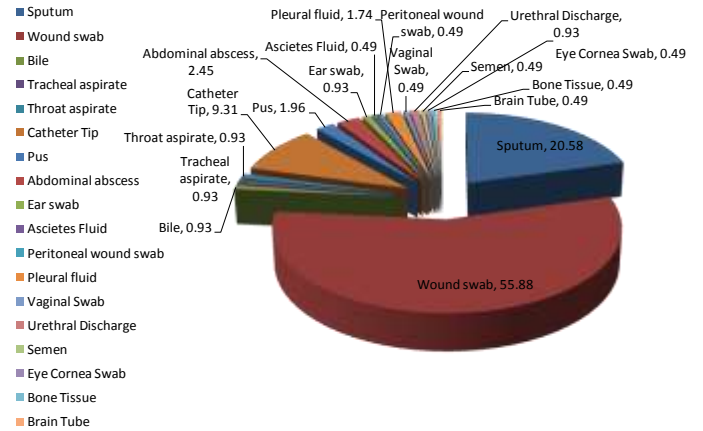

Figure 2. Percentage of isolates found positive for *E. coli* in males and females**.**

Figure 3. Percentage of *E. coli* specimens isolated from different clinical samples.

resistant to almost all available antimicrobials leading to many casualties each year (Ventola, 2015). Several studies have been reported on antimicrobial resistance patterns in *E. coli* from KSA (Rotimi et al., 1998; Al-Johani *et* al., 2010; Halawani, 2011; Zowawi, 2016) but none has been reported from Madinah, one of the two important cities visited by many pilgrims all year round. The present study is an attempt to study the antimicrobial resistance pattern of *E. coli* isolated from patients at King Fahad Hospital, Madinah, KSA. Exactly 6840 samples were collected from clinical sources over a period of 14 months and screened for *E. coli*. Results show that in comparison to other clinical isolates, only 3.0% *E. coli* strains were isolated (Figure 1). No *E. coli* isolate was recovered from some samples including urine, blood, ascetic fluid, nasal swabs, axilla, and perineum. Of the positive isolates, 71.6% were from clinical samples of male patients while 28.4% were from females (Figure 2). A similar study done in Makkah has also recorded a

higher percentage of isolates from males (Haseeb et al., 2016).

Figure 3 shows the percentage of *E. coli* strains that could be retrieved from various sources. Majority of the *E. coli* strains were isolated from wound swabs (55.88%) and sputum samples (20.58%) followed by catheter tips (9.31%). While 2.45, 1.96 and 1.74% of *E. coli* isolates were recovered from abdominal abscess, pus and pleural fluid samples respectively. For the remaining clinical samples, less than 1% were recovered in bile, tracheal and throat aspirates, ear swabs, urethral discharge, ascites fluid, peritoneal wound swab, vaginal swabs, semen, eye cornea swabs, bone tissue and brain tube. Table 1 gives a gender-wise estimation of the number of male and female samples isolated from different sources. In wound swabs and sputum samples, 61.9 and 66.8%, respectively were obtained from males. Only 19 *E. coli* strains were isolated from catheter tips wherein 16 were from males and only 3 were from female patients. The male to female ratio in abdominal abscess, pus and pleural fluid were 4:1, 2:2 and 2:1, respectively. High isolation rates from sputum and wound specimens have been reported earlier also (Masoud et al., 2011; Kibret and Abera, 2011).

As described previously, the gender-wise distribution of the samples revealed that in general, greater number of *E. coli* strains were isolated from males which may be indicating that adult males are more susceptible to infection than adult females (Haseeb et al., 2016; Magliano et al., 2012). The results can be elucidated on the basis of different lifestyles and socio-economic conditions of the patients. Since the males constitute a larger workforce in Saudi Arabia, it is not surprising that greater samples were obtained from males than females. Only 1 sample each was available from ascites fluid, peritoneal wound swab, vaginal swabs, semen, eye cornea swabs, bone tissue and brain tube.

Antimicrobial drug susceptibility assay was performed

Table 1. Gender-wise distribution of *E. coli* specimens isolated from different sources.

Sex	Sp	WS	Bile	Tr	Th	Cath	Pus	Abd	Ear	AF	Peri	Pler	VS	UD	Semen	ECS	Bone tissue	Brain tube
M	30 (66.8)	82 (61.9)	1 (50)	2 (40)	1 (66.7)	16 (82.1)	2 (50)	4 (80)	1 (50)	1 (100)	0 (0)	2 (66.7)	0 (0)	2 (100)	1 (100)	0 (0)	0 (0)	1 (100)
F	12 (33.2)	32 (38.1)	1 (50)	0 (60)	1 33.3	3 (17.9)	2 (50)	1 (20)	1 (50)	0 (0)	1 (100)	1 (33.2)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)
Total	42	114	2	2	2	19	4	5	2	1	1	3	1	2	1	1	1	1

M, Males; F, Females; Sp, Sputum; WS, Wound swab; Tr, Tracheal aspirate; Th, Throat aspirate; Cath, Catheter Tip; Abd, Abdominal abscess; AF, Ascietes Fluid; Peri, Peritoneal wound swab; Pler, Pleural fluid; VS, Vaginal Swab; UD, Urethral Discharge; ECS, Eye Cornea Swab. Percentage (%) values are given in parentheses.

Table 2. Percentage (%) of antimicrobial sensitivity pattern of *E. coli* specimens to different antimicrobials.

Antibiotic	Sensitive	Resistant	Intermediate
Ampicillin	87.5	12.5	0.0
Augmentin	91.1	8.9	0.0
Gentamycin	98.6	1.4	0.0
Cefoxitin	98.7	1.3	0.0
Cephalothin	91.4	0.6	0.0
Cotrimoxazole	92.3	7.7	0.0
Amikacin	100.0	0.0	0.0
Ceftazidime	98.7	1.3	0.0
Aztreonam	100.0	0.0	0.0
Piperacillin	95.7	4.3	0.0
Imipenem	100.0	0.0	0.0
Ciprofloxacin	98.7	1.3	0.0

using antimicrobial discs of ampicillin, augmentin, gentamycin, cefoxitin, cephalothin, cotrimoxazole, amikacin, ceftazidime, aztreonam, piperacillin, imipenem and ciprofloxacin and the results are shown in Table 2. The antimicrobials imipenem, aztreonam and amikacin were the most effective drugs against *E. coli* strains with 100% sensitivity followed by ciprofloxacin, ceftazidime and cefoxitin with 98.7% sensitivity. Gentamycin was also effective with a sensitivity of 98.6%.

Amikacin and aztreonam are treatment options for infections caused by bacteria belonging to carbapenem-resistant enterobacteriaceae (CRE) group (Bader et al., 2017). Imipenem has been highly effective against Gram-negative bacteria as shown by several other studies (Mohammed et al., 2016; Bahashwan and Shafey, 2013; Dash et al., 2014; Alam et al., 2017). Aztreonam and ciprofloxacin have been recommended as better antimicrobials against *E. coli* (Kirac et al., 2016).

The percentage resistance to the antimicrobials used in the present study was not very high and was in the following sequence: Ampicillin (12.5 %) > Augmentin (8.9 %) > cotrimoxazole (7.7 %) > piperacillin (4.3 %) > gentamycin (1.4 %). Cefoxitin, ceftazidime and ciprofloxacin showed percentage resistance of 1.3% while amikacin, aztreonam and imipenem showed no resistance at all. The antimicrobial cephalothin showed a low resistance of 0.6% only. The results are

Table 3. Percentage (%) of *E. coli* infections pattern during different seasons.

Season	Percentage (%) of *E. coli* infections
Summer (22 June -22 September)	30.2
Autumn (23 September -21 December): Pilgrimage season	21.6
Winter (22 December -30 Mars)	30.9
Spring (21 Mars-21 June)	17.3

consistent and in compliance with previous studies (Inan and Gurler, 2004; Kirac et al., 2016). Attention should be given while prescribing cotrimoxazole, Augmentin, and ampicillin to avoid increasing resistance patterns by *E. coli.* They should be used in life threatening mutidrug resistant infections where there is no other alternative. In general, prescription for infection treatment should be based on WHO's critically important antimicrobials for human medicine.

Seasonal variations are commonly observed while studying the incidence of bacterial infections. These seasonal trends are influenced by several factors which can be identified by exploring their prevalence in detail (Fares, 2013). With the help of this and similar studies, novel and improved infection control strategies can be formulated. Several reports claim that bacterial infections always peak during summers and winters (Perencevich et al., 2008; Eber et al., 2011; Richet, 2012). Table 3 illuminates the *E. coli* infection pattern during four different seasons of the year in Madinah. Infections occurred with a higher and similar frequency in both summers (30.2%) and winters (30.9%). In the intermediate seasons, that is, autumn and spring, when the temperatures are not extreme, the percentage of *E. coli* infection reduces significantly to 21.6% in autumn and 17.3% in spring. During this period, the autumn season coincides with the annual pilgrimage called Hajj when a huge population of pilgrims visits this city. The reason for the decline in the percentage of infection may be due to the efforts of the health care workers in that period as the health authorities take special precautions in controlling and monitoring outbreaks of different microbes. A sudden rise in the percentage of infection cases after 21[st]June when summers start is not surprising. Similarly a sudden rise can be seen after 21[th]December when winters begin is also reported earlier (Richet, 2012). Similar patterns have also been observed with other gram negative bacterial species of *Proteus* (Bahashwan and Shafey, 2013), *Klebsiella* (Ghanem et al., 2017) and *Pseudomonas* (Saeed et al., 2018) during the same period of study.

Saudi Arabia has to face several challenges to keep both infections and MDR in control especially in the two holy cities. There is an influx of pilgrims throughout the year but it is during the time of the annual pilgrimage (Hajj), the cities are vulnerable to epidemics. But interestingly, during this season which coincides with autumn, increase in percentage of *E. coli* infection was not observed. Implementation of the World Health Organization (WHO) hand hygiene program and the Gulf Cooperation Council (GCC) Infection Control Program (Yezli *et* al., 2014) are some of the good initiatives taken by the Saudi government in controlling spread of resistant pathogens in healthcare units. Another program that helps in reducing MDR is the antimicrobial stewardship program (Alawi and Darwesh, 2016; Zowawi, 2016).

Conclusion

Wound swabs followed by the sputum samples turned out to be the largest source of *E. coli* isolates. Samples from male patients were greater in comparison to female patients, maybe because males are at a larger risk to infection. The antimicrobials imipenem, aztreonam and amikacin showed 100% sensitivity. These infections occurred with a higher frequency in both summers and winters but the infection percentage dropped during intermediate seasons. To limit the inappropriate use of antimicrobials and control the spread of MDR, there is a need of active surveillance, creating awareness in the medical community and changing the attitude and prescribing habits of physicians. New guidelines and awareness programs should be formulated and strictly followed. More and more studies should be done on MDR and sensitivity pattern of antimicrobials. Studies like this will help in developing better infection control policies and generate local databases for infection control strategies within this region.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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