

The Study of the Relationship between the Dominant Bacterial Species and Antimicrobial Susceptibility Tests Derna-East Libya

Mabroka H. Tarfaya, Mona A. El-mahdi, Zahra M. Bomousa

Abstract: Background: When focusing on Low-middle income countries there need to highlight understand the inhibitory effect of an antimicrobial drugs on antimicrobial resistance bacterial strain (AMR), and multi drug-resistant bacterial strains (MDR), in fact in, were there the misused of antimicrobial drugs, and the delay of update generation of antimicrobial drugs if compared with speed of gene mutation of the antimicrobial resistant bacterial strains to drugs. Therefore, that study included the dominant bacterial strain and rate of resistance to antimicrobial drugs. Methods: This study was carried out in the microbiology department at Al-Wahda teaching hospital - Derna-East Libyan state, for two years ago from January 2015 to December 2016, was collect 1326 patient samples from almost hospital wards surgical, medical, obstetrics and gynecology, pediatrics, isolation, neonatal care, intensive care unit, outpatient department, and from deferent site of the body and body fluids like (urine, stool, pus, throat), all age groups, and from both sex, was cultured isolate bacteria on ordinary medium and antimicrobial susceptibility test was done by a diffuse technique. Findings: The first once in Al-Wahda hospital were collect date analysis for determination of dominating bacterial strain with study the inhibitory effect of antimicrobial drugs on this strain, were objected to 1326 samples were had almost commonly samples 980(73.96%) urine, the study was conducted on 18 species of pathogenic bacterial strains and non-pathogenic, We're was classified growth depend on gram reaction, were observe result not significant, were was 305 (47.12 %) gram-positive bacteria and 287 (44.427%) gram-negative bacteria. The total Antimicrobial susceptibility test was done for pure isolate colony to 646 (48.72%) samples are 70322 tests for gram-negative 30422(43%) tests, and for gram-positive 39900(57%) tests from pure isolated colonies, we studied the effected of potency of antimicrobial drugs on Gram Negative, and positive bacterial strain concerning eight antimicrobial groups (Quinolones, Carbapenems, Penicillin's, Cephalosporin's, Aminoglycosides, Glycopeptides, Sulfonamides, Lincomycin derivatives, observed the carbapenem (mainly meropenem) are the strongest of antimicrobial susceptibility inhibitor rate on most bacterial species, Where the percentage of inhibitory effect on gram-negative bacteria strain 90%, and 75% on gram-positive strain with good effect on MRSA, MRS, ESBL, AmpC B.lactamase Production species. and Were didn't record resistance cases to Carbapenem group until now. Interpretation: These data are essential for the development of antimicrobial stewardship programmers, are needed to assess local susceptibility patterns and help in selecting an empiric antibiotic therapy and in monitoring resistance trends in the hospital. And due to the high prevalence of multidrug-resistant (MDR) and antimicrobial-resistant bacteria species (AMR), There is a need for the development of strong policies on antibiotic stewardship, antimicrobial surveillance and infection control to prevent the spread of AMR bacteria strain and MDR multi-drug resistance. Funding: Nosocomial infection Prevention Control Committee- Derna -Libya (NIPCCD) and members of Microbiology department at Al-Wahda Teaching Hospital

1. Introduction

Fundamentally, the resistance of bacteria to antimicrobial drugs is expected occurs in high -middle - low-income countries. the reason are easy to access to get of antimicrobial drugs also led to their misuse and overuse,^{15,17,21} that could be due to we did not make antibiotics on the list of use prohibitions from the baseline, and this arranges it the emersion of the new bacterial mutations resistant to antibiotics. the spread of this strain threat the wide world, prompting bacteria to develop resistance.^{22,17}, prompt the World Health Organization to classify antimicrobial resistance as a "superbugs threat the world, that is a disaster for the future, it is happening in every region of the world and has the potential to affect anyone, of any age, in any country."²⁰

Threatens the stability of the world from superbugs new resistance mechanism that reduces your ability successes to prevent infectious disease, that lead to prolong illness, increase the rate of morbidity and mortality, without effectiveness of antimicrobial drugs to prevent and treat of infection, medical procedures such as organ transplantation, cancer chemotherapy, diabetes management, and major surgery (for example, caesarean sections, or hip replacements) become very high risk, Antimicrobial resistance increases the cost of healthcare with prolongs illness time in hospitals and more intensive care required.³ Antimicrobial resistance is putting the gains of the

Millennium Development Goals at risk and endangers the achievement of the Sustainable Development Goals.³

Realistically, in low - middle-income countries the Clinical microbiology laboratories need to communicate the results of an antibiotic susceptibility testing to prescribers as soon as possible; like any good clinical microbiology laboratories in high-income countries. The fastening communicates of the antibiotic susceptibility testing results contribute to getting the best antibiotic prescription and give a good prognosis. The goals of treatment of any infections depended on the use of appropriate antimicrobial agents directed against the pathogen.¹ In the absence of microbiology laboratory facilities for the isolation, identification and an antimicrobial susceptibility testing, the prevailing an antibiotic susceptibility patterns in the regions usually serve as the clinical guide in the choice of the appropriate antimicrobial drugs.¹ Therefore unable use all antibiotic for all types of infection. So, the susceptibility of the organisms to the antibiotics needs to be checked.⁴ The choice of antibiotics to treat simple infections can usually be made based on historical data and the clinician's experience.¹⁹ The antibiotic used is too broad and unnecessary.

Actually, the antimicrobial resistant bacteria strain and multi drugs resistance to antimicrobial drugs; have been observed with increasing frequency over the past several decades.¹⁸ especially in wounds infection cases mainly after an operation, after Cesarean section, or due to the gunshot

injuries, and other the war injuries, or bedridden patient, and after invasive devices such as catheters.

All of that enables an increase in the coming years, If not resisted. coincide with the increased bacterial resistance there has been waning antibiotic development.¹⁸

The first one of bacterial resistance to antibiotic drugs to treat infections caused by *Staphylococcus* a common cause of severe infections in health facilities and the community known as methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus coagulase negative* (MRS), that are more resistant to antimicrobial drugs a widespread in our region like any area in the world. are estimated to be 64% more likely to die than people with a non-resistant form of the infection.⁹ In Americans Just one organism, methicillin-resistant *Staphylococcus aureus* (MRSA), kills more Americans every year than emphysema, HIV/AIDS, Parkinson's disease and homicide combined.¹² ESBL Increasing prevalence of multidrug-resistant MDR Gram-negative bacteria has continuously been reported over the past years, In particular, the Enterobacteriaceae producing extended-spectrum B.lactamases. ESBLs that enable to hydrolyze penicillins, first- second, and third-generation cephalosporins, and aztreonam but not effect on cephamycins, and carbapenem groups.¹⁶ ESBL producing organisms can be responsible for life-threatening infections, that leading to increased the morbidity, mortality and healthcare-associated costs. A fast and accurate detection of ESBL carrying the Enterobacteriaceae strains is needed to prevent the complication ESBL producing strain.¹⁶ all that enhanced cost of patient management due to prolonged hospitalization.²³ prompt detection, identification, and confirmation of these strains by clinical laboratories has a lot of infection control implication of rapid appropriate control measures to prevent its further spread in the hospital.²³

Therefore, the discussion hub collects between the rate of bacterial resistance to antimicrobial drugs and complain about feedback of prescription drugs without a guideline, to reach how management that in the low middle-income country.

2. Materials and Method

Samples collection

This study was carried out in the microbiology department at Al-wahda teaching hospital - Derna-East Libyan state for two years ago from January 2015 to December 2016, was collect 1326 patient samples from almost hospital wards surgical, medical, obstetrics and gynecology, pediatrics, isolation, neonatal care, intensive care unit, outpatient department, and from both sex, and average age. this sample was collected for ordinary investigation for admitted patient and from outpatient department for culture and antimicrobial susceptibility testing AST, Types of samples depended on types of infection urine, pus, wound infection, stool, cerebrospinal fluid CSF, synovial fluid, and some quality control samples getting from the operation room, delivery room, and intensive care unit.

Medium and Antimicrobial Susceptibility Testing AST

Low-middle-countries microbiology laboratories different from where the facility of The possibilities, quality, accuracy, and evolution of microbiology laboratories in high-income countries, but correspond to one guideline according to the national committee for clinical laboratory standers (NCCLS) to achieve the best goals.

All samples were cultured on ordinary media Blood agar, Chocolate agar, Cystine Lactose Electrolyte-Deficient Agar CLED, MacCongy agar, Salmonella Shighelia SS agar depended on the types of samples and identified of isolate significant colony by gram stain and some special biochemical test like Catalase test, Coagulase test, or by the hemolytic zones were characterized as α (partial hemolysis), β (complete hemolysis), and γ (no hemolysis) depending on the extent of each colony², and some of the biochemical test for Enterobacteriaceae species like Triple Sugar Iron Agar (TSI), Kligler Iron Agar (KIA), and Lysine-Iron-Agar (LIA).

In high-income country use the new method for differentiated and identification of MRS, MRSA these include quantitative *mecA* PCR procedures,⁵ rapid agglutination latex method penicillin-binding protein PBP2a,⁵ or newly by CHROMagar™ MRSA II* is a selective and differential medium for the direct detection of methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical specimens.⁶ In our laboratory (low-middle-income country), we identified by a simple procedure like hemolytic activity and coagulase test, the oxacillin resistant, The cefoxitin, the vancomycin disk diffusion method can be used in addition to routine susceptibility test methods and ESBL detection involves two important steps; The first is a screening test with an indicator cephalosporin which looks for resistance or diminished susceptibility, thus identifying isolates likely to be harboring ESBLs. The second step is a confirmation test which evaluates the synergy between an oxyimino cephalosporin and clavulanic acid, distinguishing isolates with ESBLs from those that are resistant for other reasons, Enterobacteriaceae suspected to be producers of ESBL enzymes may be confirmed by evaluating the inhibition of ESBL activity by Clavulanic acid. The inhibition zone around the cephalosporin disc combined with clavulanic acid is compared with the zone around the disc with the cephalosporin alone.⁷ AmpC's and carbapenemases resistant bacteria strain can be indicted by antimicrobial susceptibility test.

Disk Diffusion Technique

Al-Wahda teaching hospital -Derna have used stander disc diffusion technique for antimicrobial susceptibility test according to the national committee for clinical laboratory standers (NCCLS2004)⁸, and updating depended on possibility available. Kirby-Bauer disc diffusion test method is a reference method which could be used as a routine technique to test the sensitivity of the isolate in the clinical laboratory. The disc diffusion method was originally described in 1966, and is well standardized and has been widely evaluated³³. Kirby-Bauer test, known as the disk-diffusion method, is the most widely used antibiotic susceptibility test in determining what choice of antibiotics should be used when treating an infection. This method

relies on the inhibition of bacterial growth measured under standard conditions. For this test, a culture medium, specifically the Mueller-Hinton agar and some strain isolated on Blood-Muller Hinton agar as a special environment for antimicrobial susceptibility test by a diffusion technique method Kirby Bauer method, is uniformly and aseptically inoculated with the test organism and then filter paper discs, which are impregnated with a specific concentration of a particular antibiotic, are placed on the medium. The organism will grow on the agar plate while the antibiotic "works" to inhibit the growth.³² If the organism is susceptible to a specific antibiotic, there will be no growth around the disc containing the antibiotic.³¹ Thus, a "zone of inhibition" can be observed and measured to determine the susceptibility to an antibiotic for that particular organism. The measurement is compared to the criteria set by the National Committee for Clinical Laboratory Studies (NCCLS). Based on the criteria, the organism can be classified as being Resistant (R), Intermediate (I) or Susceptible (S).³¹ This method most commonly used in the wide world 'the Manual methods that provide flexibility and possible cost savings include the disk diffusion and gradient diffusion methods'³⁴ therefore in our microbiology laboratory used this technique for AST

Data collection and analysis

Data collection and analysis by conventional criteria, this difference is considered to be extremely statistically significant by measure mean and p-value for differentiation between dominant bacteria strain gram-positive and Gram-negative, calculate the highest percentage of potency effect of antimicrobial susceptibility test on the strain, and study which one more effect by antimicrobial drugs and lesser effect on the bacteria in vitro diagnosis

3. Results

The antimicrobial susceptibility patterns of bacteria isolated from hospitalized patients varies significantly throughout hospitals in low-middle income countries. The routinely collect antimicrobial susceptibility data, and determination of dominant strain in the daily workload on Al-Wahda teaching hospital is the first time registered. Wherefore we collect stratification of data for 2 years ago from January 2015 to December 2016, and continuous collect samples and data for coming years to get good responses for good management of AMR. In this studies determine types of microbial species, and antimicrobial susceptibility choices the collected data among 2015 and 2016. Includes number and types of samples, types of microbial species, antimicrobial potency in vitro diagnosis, and a relation between types of dominating bacterial growth with an inhibitory effect of antimicrobial susceptibility.

The samples studied was 1326 patient samples from almost hospital wards surgical, medical, obstetrics and gynecology, pediatrics, isolation, neonatal care, intensive care unit, and from the outpatient department.

The samples were collected from deferent sites of the body and body fluids like (urine, stool, pus, throat, synovial fluid, cerebrospinal fluid) were had on this study almost commonly samples 980(73.96%)urine, 29(2.19 %)stool

samples, 41(3.09%) throat swabs, 5(0.38 %)pus samples, 105(7.92%) CSF, wound infection samples 22(1.66%), 142(10.71 %) quality control samples from intensive care patient, and instrument, and not significant percent from other body fluids like synovial 1(0.075%), saliva 1(0.075%), and 0% Blood culture samples due to not available in our laboratory ;table1

We're was studied on 1326 samples, then classified of laboratory result depended on the colony number of microorganism growth, first class significant growth more than 10^3 , or 10^5 microorganisms each species of growth 646(48.72%), second class not significant growth or no growth less than 10^3 , or 10^5 microorganism depending on types of samples 552(41.66%), and third class mixed bacterial growth 128(9.58%) more than 2 species of microorganism growth could be defect, or contamination during collection samples; table 2

There has been undergoing to studies 18 strain from the dominant bacterial strain to gram positive and negative bacteria in 646 (48%) from total samples, there were 54 (8.359%) from growth as normal flora or commensal growth, 305 (47.12 %) gram-positive bacteria and 287(44.427 %) gram-negative bacteria; figure 1

The total Antimicrobial susceptibility test to 646 samples are 70322 tests for gram-negative 30422 tests (43%), and for gram-positive 39900 tests(57%) from pure isolated colonies. Where was studied the inhibitory effect of the antimicrobials drugs potency in vitro diagnosis on the types of dominant gram-positive strain in our hospital, Where was studied the inhibitory effect of the antimicrobials drugs potency in vitro diagnosis on the types of dominant gram-positive strain in our hospital, When found most strain different from each other in terms of the grade of impact of antibiotics potency, the reported better effect of antimicrobial susceptibility tests (AST) their Streptococcus species rather than catalase positive staphylococcus species, the antimicrobial potency effect on Streptococcus pneumonia group. B 73 %, Beta. hemolytic streptococcus 57.89 %, Streptococcus pyogenus 53.84 %, Enterococcus species and for Staphylococcus species were found coagulase-negative Staphylococcus Saprophyticus 53.33 %, Staphylococcus epidermatis 50.00%, and coagulase positive Staphylococcus aureus 44.40%. and Oxacillin/Methicillin-resistant coagulase positive Staphylococcus aureus (MRSA) 18.18 %, Oxacillin-resistant Coagulase-negative Staphylococcus (MRS) Species 18.18 %, the normal flora, and commensal growth; Logically not be tested for AST and Candida Albicans are significant as a gram-positive reaction mycology only; figure 2. Where was studied the relationship between the antimicrobials drugs potency in vitro diagnosis with the types of dominant gram negative strain in our hospital, where found most strain effective for antimicrobial susceptibility testing (AST), was Morexalla 77.78%, Acinetobacter baumannii 75%, Escherichia Coli species 58.82%, Nisseria Meningitidis 77.78%, 50.00%, Pseudomonas Speices 46.15 %, klebsellia species 45.45%, proteus species 45% and ESBL 13.13% Extended-Spectrum Beta-Lactamases ;figure 3. When we studied the effected of potency of antimicrobial drugs on gram-positive bacterial strain, and gram-negative bacterial strain for 8 antimicrobial

groups (Quinolones, Carbapenems, Penicillin's, Cephalosporin's, Aminoglycosides, Glycopeptides, Sulfonamides, Lincomycin derivatives, were found the stronger one on gram-positive bacterial strain are carbapenem group 75 % the flowing group Glycopeptides 50 %, Cephalosporin's 45%, Penicillin's 43 %, Quinolones 30%, Lincomycin derivatives 30%, Aminoglycosides 22.70%, and Sulfonamides 14.20%; figure 4. And When we studied the effected of potency of antimicrobial drugs on gram Negative bacterial strain for 8 antimicrobial groups (Quinolones, Carbapenems, Penicillin's, Cephalosporin's, Aminoglycosides, Glycopeptides, Sulfonamides, Lincomycin derivatives, were found the stronger one on gram-positive bacterial strain are carbapenem group 90 % the flowing group Quinolones 57%, Aminoglycosides 50 %, Cephalosporin's 50%, Lincomycin derivatives 27%, Penicillin's 25 %, Sulfonamides 12,50 % and Glycopeptides 0 %; figure 5

4. Discussion

Libya state, that one of African low - middle-income countries, which loss of application of protocols guidelines used of antimicrobials drugs in the hospitals, where we discuss one of the hospital's experience. Our hospital assessed antibiotic patterns and collected information about antibiotic resistance in the microbiology department for the first time. In addition to the identification of endemic bacterial patterns in the study area(Derna east Libya), where the study was conducted on 18 species of pathogenic bacterial strains and non-pathogenic, These data are essential for development of antimicrobial stewardship programmers, WHO AMR antimicrobial resistant plain, Nosocomial infection Prevention Control Committee- Derna - Libya(NIPCCD) and National Centre for Disease Control - Libya(NCDCL) are needed to assess local susceptibility patterns and aid in selecting empiric antibiotic therapy and in monitoring resistance trends in the hospitals. and due to the high prevalence of multidrug-resistant (MDR) and Antimicrobial resistant bacteria species(AMR), There is a need for an action plan to development of strong policies on antibiotic stewardship, antimicrobial surveillance and infection control to prevent the spread of antimicrobial resistant bacteria strain AMR, and multi-drug resistance MDR. This study we conducted was compared with another perspective study going to similar goal carried out by The Global Point Prevalence Survey (Global-PPS) established an interface worldwide team in 53 countries around the world and 303 hospitals. In the our study showed the carbapenem are the antibiotic group with highest rate of susceptibility broad spectrum effect on the most bacterial species from gram positive and gram negative bacterial strain Where the proportion of its effect on 90% gram-negative bacteria strain and 75% on gram-positive strain with good effect on MRSA, MRS, ESBL, AmpC B.lactamase Production species. and We have not recorded cases of resistance to Carbapenem group until now, if compared with the results of the study carried out by The Global Point Prevalence Survey (Global-PPS) Carbapenems (mainly meropenem) were widely prescribed in Africa countries flowing Fluoroquinolones and fourth generation of cephalosporin,¹⁰ and the carbapenems (imipenem and meropenem) demonstrated the highest susceptibility rates ($\geq 98.7\%$) and with only a 1% increase in

resistance for ICU isolates compared to non-ICU organisms. Other antimicrobial agents tested demonstrated consistently higher susceptibility rates against Enterobacteriaceae isolates from ICU (89.7–98.7%) and non-ICU (93.2–99.9%) areas.²⁹

Which is in line with the high proportion of patients who received targeted treatment against MRSA infections in our survey hospital. The prescription of carbapenems should be restricted to the initial management of serious bacterial infection in which MDR or AMR species (MRS, MARSA, ESBL, AmpC BL production) are suspected and should be avoided in situations where a narrow spectrum antibiotic would be equally effective.

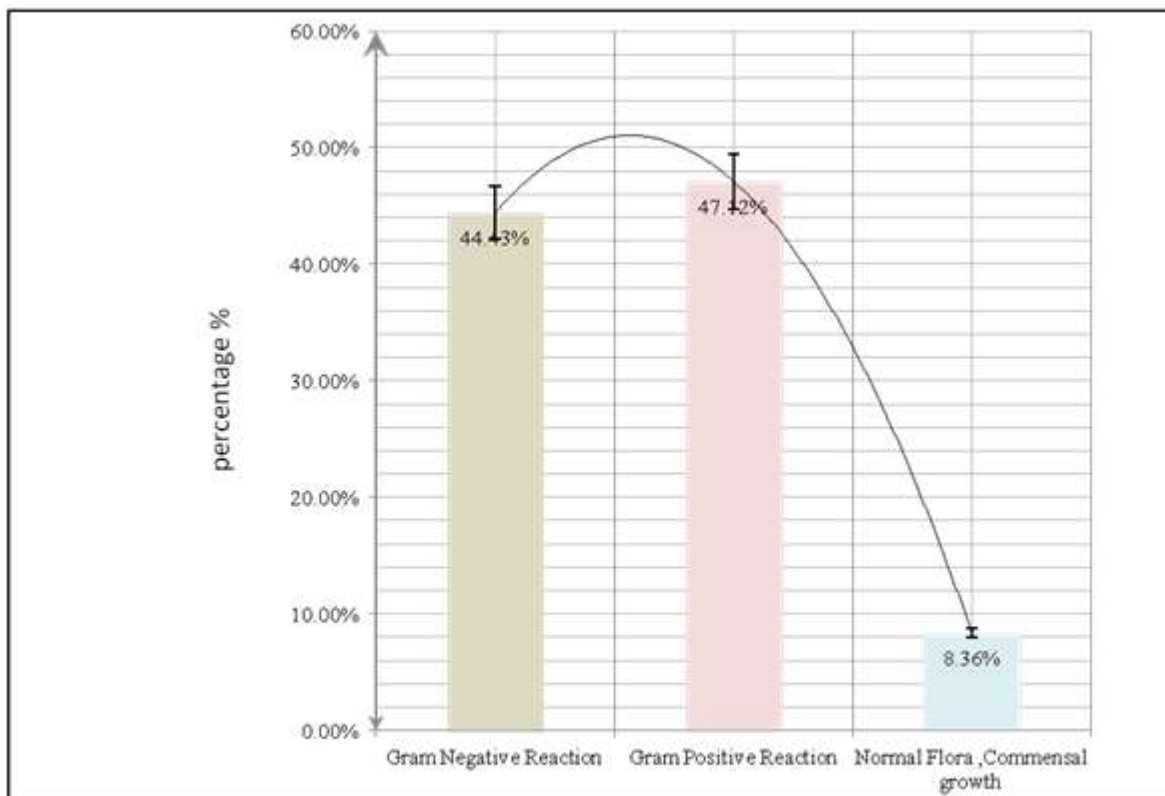
The stories today are broader in scope and more frequent. In the United States,³⁰ At least 30% of antibiotics prescribed in the United States are unnecessary,²⁸ and the percentage of antibiotics unnecessarily prescribed in the rest of the world is estimated to be even higher. The Centers for Disease Control and Prevention (CDC) released a report in 2013 estimating that 2 million Americans become ill every year from resistant infections and 23,000 die from them (CDC 2013). Over the past decade, the World Health Organization (WHO) has issued several reports that have drawn some attention, but the global discussion still lacks a strong voice from low- and middle income countries, where the problems and solutions differ in some important ways from those in high-income countries.³⁰ the Global Antibiotic Resistance Partnership (GARP) model ReAct-assisted model have had successes on individual country levels. The WHO has intermittently provided effective efforts at combating antibiotic resistance in the last decade.³² New resistance mechanisms are emerging and spreading globally, threatening our ability to treat common infectious diseases, resulting in prolonged illness, disability, and death.⁹ Awareness of the need to curb antibiotic use is on the rise globally, but policies and actions are more prevalent in high-income countries. As incomes in low- and middle-income countries rise, antibiotic use increases, but there are still significant problems concerning lack of access to antibiotics in such place. At the same time, overuse of antibiotics is occurring in other areas of these same countries.³² In fact, according to our recognized records, suffering began the Since 2011 with the emergence of strains resistant to antibiotics in many cases of wounds infection. The importance of strengthening AMR surveillance in low-income countries was focused in 2014 by a United Kingdom government-commissioned review.²⁴ In response, the United Kingdom Department of Health launched the Fleming Fund to support low-income countries in developing AMR surveillance systems.²⁷ The fund is aligned with the World Health Organization (WHO)'s Global AMR Surveillance System (GLASS)²⁶ Food and Agriculture Organization (FAO), And World Organization for Animal Health(OIE) support the Global Action Plan on AMR.²⁵ The aims of the WHO AMR surveillance programmed include monitoring trends in infection and resistance to develop standard treatment guidelines that support best practice for patient care, In one African low-middle income countries, 11% of patients were treated with antibiotics for an unknown diagnosis, contrary to guidelines for that state an appropriate treatment must be preceded by diagnoses that

ensures the correct clinical path.¹¹ Correct diagnosis and treatment planning requires the existence of a clinical microbiology laboratory and antimicrobial stewardship involvement. Next to developing and updating local treatment guidelines, adherence to guidelines could improve clinical outcomes (mortality, treatment duration, and prolonged hospital stay, and a decrease of therapy cost).¹³ Actually, there is no doctor in low- and middle-income countries do not prescribe at least one antibiotic for any patient who benefits or does not benefit without the guideline. Also not taking in consideration during antimicrobial prescriptions of the age grouping, site of infection (systemic, or not systemic), stages of pregnancy, types of wound infection (deep, or superficial), as well as route of drug administration, clinical pharmacy intervention, cost-effectiveness with probability of treatment failure, and guidelines implementation all that taking in your account when choosing the most appropriate antibiotic for the patient. There had defect or gap between antimicrobial susceptibility testing and clinical pharmacies availability of antibiotic, so laboratory results didn't have medical significant for many cases, however, the doctor could be taken randomly decision to choose antimicrobial drug dependent on whatever available. Without considering the possibility of bacterial resistance to the antibiotic used and what are the consequences of creating new mutation of bacterial resistance to antimicrobial drug. documentation of the reason for prescription ensures communication of diagnosis and treatment among clinicians and other health care providers and allows for recording of prescription stop or review dates and other interventions.¹⁰ In our study, and another similar study carried out in the hospital, it was found that the most common clinical departments were the prescribed of antibiotics without recourse treatment planning necessitate the existence of a clinical microbiology laboratory and antimicrobial stewardship involvement are the medical wards, and the most committed to the applications the obstetrics and gynecology wards.¹⁴ a spread of MRSA 13.13% MRS13.13%, and ESBL 18.18% among our studies in two years 2015-2016 especially in diabetic foot and wound infection cases mainly after operation, after Cesarean section or due to gunshot and other war injuries, Can be increased in the coming years if not resisted, in fact in there the delay of update new generation of antimicrobial drugs manufactured if compared with the speed of the mutations of antimicrobial resistance bacterial strains, other causes like over-prescribed of antibiotics, lack of hygiene and Poor construction, Bad hospital building sanitation, and lack of control infection prevention protocol's management, and poorly education induction on medical and paramedical staff about infection prevention steps theirs Assisted in the development of bacterial strains to antibiotic resistant strains, the misuse of bacterial culture and susceptibility test was 41.66% no bacterial growth, and 9.58% mixed bacterial growth The likely this ratio due to was patient no need culture and susceptibility test, some doctor not depended on

routinely investigation like urine routine, could be patient had some bacterial pathogen strain not grow on ordinary culture medium, or prescribed of antibiotic before send samples for culture and susceptibility test, and for mixed bacterial growth due to lack procedure of collection or mistakes during collection samples.

The significant result p-value is <0.05 , were we compared the bacteria based on the gram reaction, where gram-positive was 47.12% and gram-negative was 44.42%. but there was more significant difference on number of susceptibility testing it was done on bacterial species was grow for gram-negative 43% and gram-positive 57% expected ratio depended on laboratory protocol. When comparing the potency effect of antimicrobial drugs on endemic bacteria, we observed that there is a relative difference effect in bacterial species if were divided into the bacteria depended on Gram reaction, that difference could be due to behavior of bacterial infection it is acquired or the opportunistic and to the extent of the ability of bacteria to resist antibiotics. The relative convergence of the effect of antimicrobials drugs groups on Gram-positive, and Gram-negative bacteria is the groups (carbapenem, cephalosporin, sulfonamide, Glycopeptides, Quinolones, Penicillins, and lincomycin derivatives) The p-value >0.05 . And the difference in the total effect of the results of antibiotic groups on the positive and negative Gram bacteria of the following groups (Aminoglycosides,) p-value <0.05 , were found 0% effect on Gram-negative and 50% on Gram-positive, also sulfonamide so latter effect on both strains for Gram-positive 14.20% and Gram negative 12.50% in vitro diagnosis figure 6 was most likely effect by antibiotic potency on Gram-positive bacteria strain streptococcus pneumonia 73 %, and more affected of antibiotic potency on Gram-negative bacteria strain Moraxella species 77.78% could be due to poorly of the spread., and It was a controversial the wound infection samples were reported as minor percentage 1.66% from total sample collected of the cases being infected and sepsis wound, There are significant differences in the actually cases numbers were admitted to hospital, which indicates that many cases of infection of the wounds are prescribed antibiotics without guideline protocols depended on the procedure of bacterial culture and susceptibility test, which requires us to deal with it to take the correct action to adjust the path of the wounds infection and sepsis cases in 2017.

What current policies are in place in low- and middle-income countries regarding antibiotic use and resistance? What related programs are in place in these countries and how effective are they? What are some policy recommendations for the future?²⁸ All these questions must be answered before it is too late.



the bacterial data collection according to gram reaction which suspects is more distributed in Derna -East -Libya

actually, are not significant results, almost equal. no big difference between Gram Positive 47.12% and Gram Negative 44.42% as a diffuse dominate strain in the region. I see no difference there worth mentioning or indicated for any new.

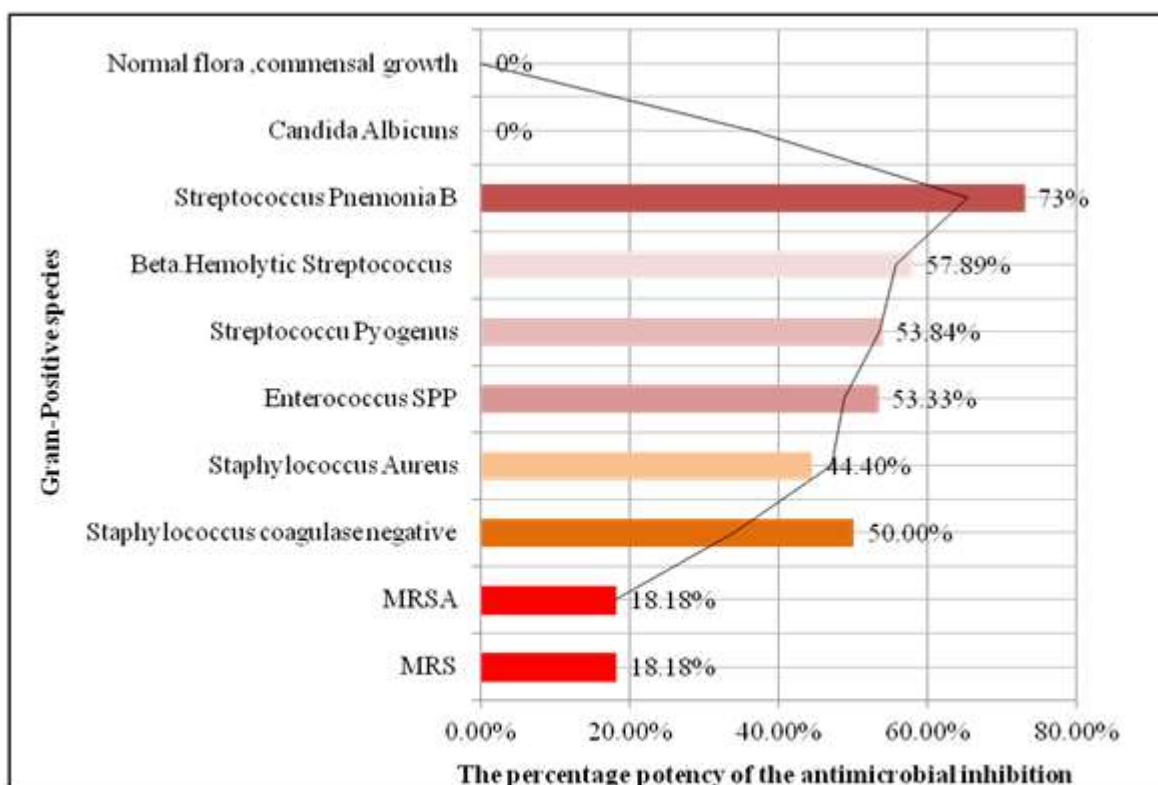


Figure 2: The grade of potency inhibitors of antimicrobials susceptibility testing on Gram-Positive Species
Normal flora, commensal growth not tested. MRSA Methicillin-resistant Staphylococcus aureus, MRS Methicillin resistant staphylococcus coagulase negative

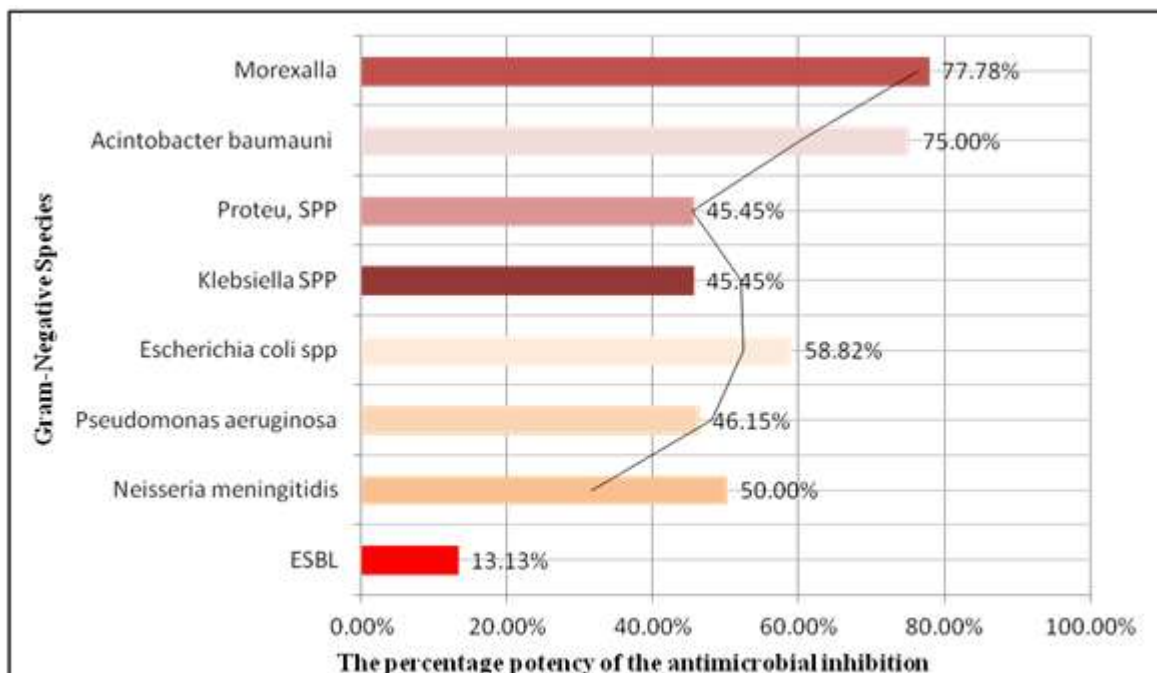


Figure 3: The grade of potency inhibitors of antimicrobials susceptibility testing on Gram-Negative Species
 ESBL Extended spectrum B. lactamase

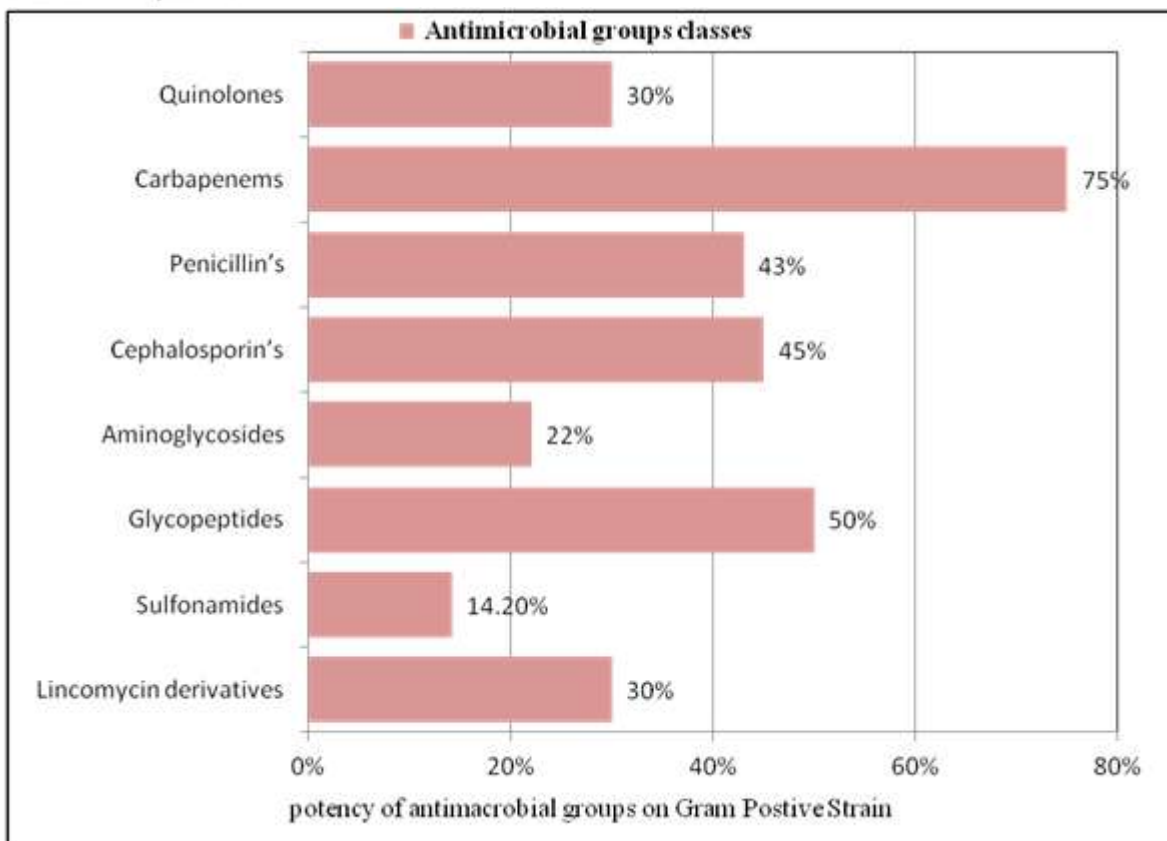


Figure 4: Describe relation between antibiotics groups and inhibitory rate on the Gram-Positive strain

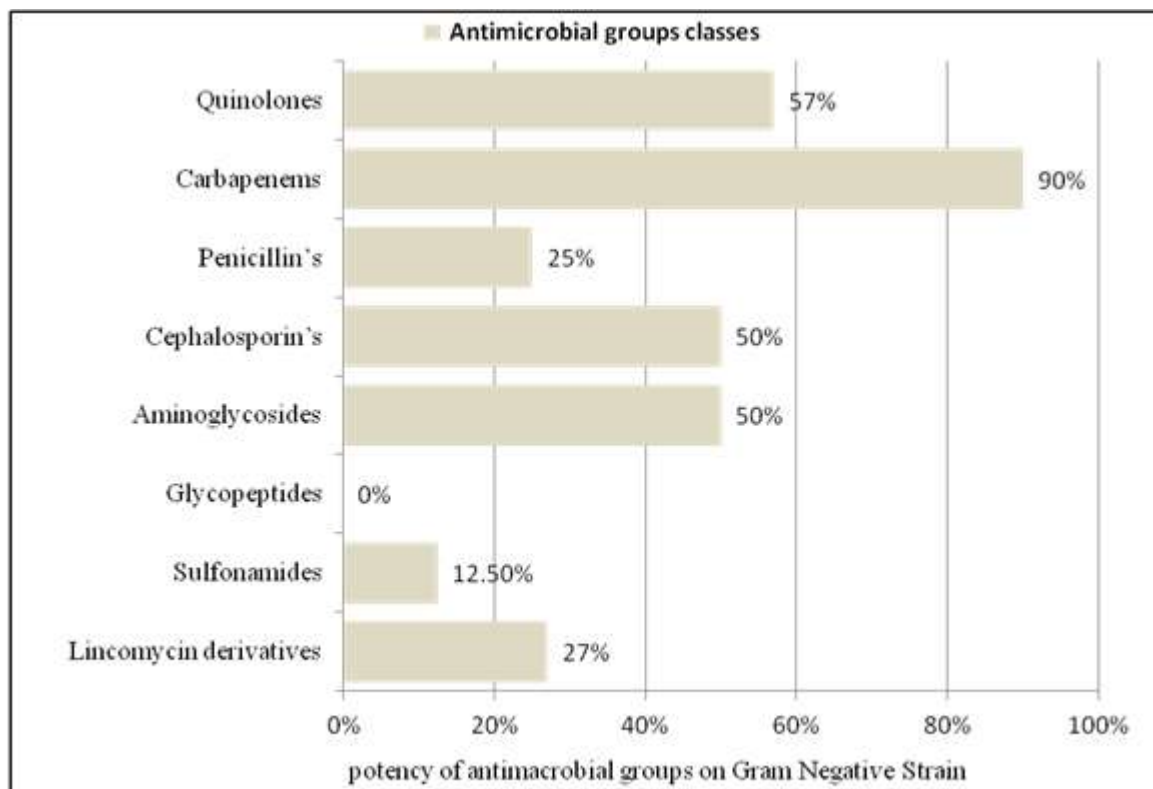


Figure 5: Describe relation between antibiotics groups and inhibitory rate on the Gram-Positive strain

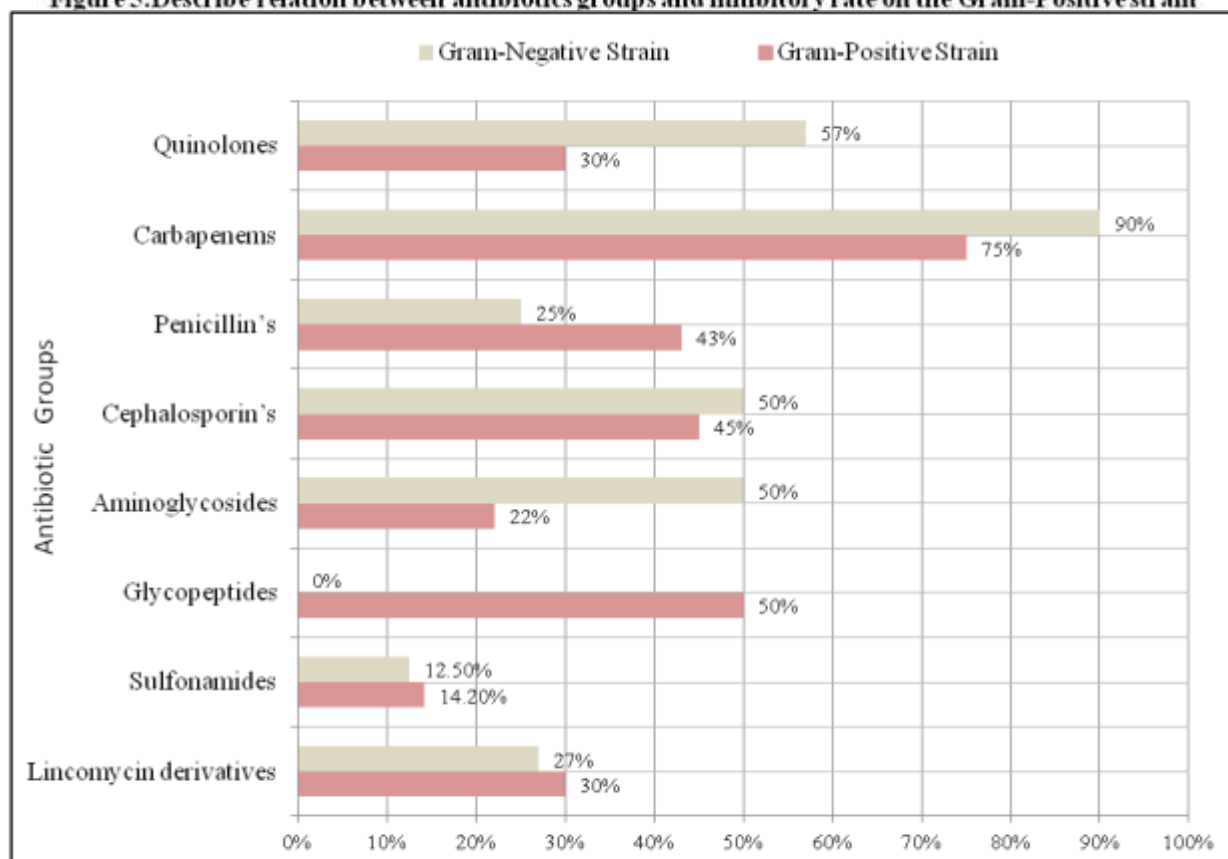


Figure 6: A comparison according to the inhibition of antimicrobial groups on Gram-positive and Negative bacterial strain

Types of samples	Number	Percentage %
urine	980	73.91%
Stool	29	2.19%
Throat	41	3.09%
Wound infections	22	1.66%
Pus	5	0.38%
cerebrospinal fluid CSF	105	7.92%
Saliva	1	0.08%
Synovial fluid	1	0.08%
Quality controls samples	142	10.7%
Blood culture	0	0%

Table1: Classification of data collection among 2015,2016 depended on types of samples

quality controls samples were collected from any place connected directly with hospitalized a patient from an instrument and equipment of intensive care unit, delivery room, and special care baby unit, to adjust dominate bacteria, blood cultures not available.

Classification of laboratories result depended on significant growth, were samples tested 1326n		
	Number	Percentage %
No, or non significant growth $<10^{3.5}$	552	41.66%
Mixed bacterial growth	128	9.58%
Significant growth $\geq 10^{3.5}$	646	48.72%

Table 2: Stratification of 1326samples depended on signification of growth

colony counts number dependent on significant of the organism causative of infection is $\geq 10^5$ Colony forming units CFU per milliliter of urine, in urinary tract infection and in stool samples, gastroenteritis infection cases, and significant growth $\geq 10^3$ colony forming units CFU per milliliter for other cases like blood, CSF, Throat, Pus, etc

5. Acknowledgment

To all the members and staff of medical laboratory and blood bank in Al-Wahda Teaching Hospital Derna Libya, so for all members of Nosocomial Infection of Prevention Control Committee Derna Libya (NIPCCDL)

References

- [1] Melecia Antonio Velmonte, Antonio J. Gonzaga, Cristina U. Darwin. *Local Production of Low Cost Quality Antibiotic Susceptibility Disks for the Philippines*, scribed ed ;1988.
- [2] Cappuccino JG, Sherman N. *Microbiology -A laboratory manual*, 5th ed. : Menlo Park, (CA);1996.
- [3] WHO. *Antibiotic Resistance*. <http://www.who.int/en/news-room/factsheets/detail/antimicrobial-resistance> (accessed 5 march 2018).
- [4] N Vineetha, RA Vignesh, D Sridhar. Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples. *International Journal of Applied Research* 2015; 1(11): 624-631(2394-5869). <http://www.allresearchjournal.com/archives/2015/vol1issue11/Part1/1-10-152.pdf> (accessed may 2018).
- [5] Centers for disease control and prevention. *Laboratory Testing for MRSA*. <https://www.cdc.gov/mrsa/lab/index.html> (accessed 5 march 2018).
- [6] BBL™ CHROMagar™ Family of Products. Germany 2009. <https://www.bd.com/resource.aspx?IDX=11164> (accessed 1 january 2015).
- [7] LIOFILCHEM®. *ESBL disc kit (acc. to EUCAST) Disc tests for confirmation of ESBL-producing Enterobacteriaceae*. Italy: Liofilchem; no date. https://www.liofilchem.com/images/prodotti-evidenza/antibiotic-disc/99004_PI.pdf (accessed 2015).
- [8] National Committee for clinical laboratory standards (NCCLS). *Performance standards for antimicrobial susceptibility testing*, 14th ed. Informational supplement: Villanova, (PA):NCCLS; 2004.
- [9] World Health Organisation. *Antimicrobial Resistance superbug the word*. <http://www.who.int/mediacentre/factsheets/fs194/en/> World Health Organization (2013) *Antimicrobial resistance. Fact sheet n°194. Updated May 2013. Available (accessed 10 may 2018)*.
- [10] Ann Versporten, Peter Zarb, Isabelle Caniaux, Marie-Françoise Gros, Herman Goossens, Global-PPS network. Antimicrobial consumption and resistance in adult hospital inpatients in 53 countries: results of an internet-based global point prevalence survey. *Lancet Global Health* JUNE 01, 2018; 6(6). [https://www.thelancet.com/journals/langlo/article/PIIS2214-109X\(18\)30186-4/fulltext](https://www.thelancet.com/journals/langlo/article/PIIS2214-109X(18)30186-4/fulltext) (accessed 6 June 2018).
- [11] Novak MT, Kotanen CN, Carrara S, et al. Diagnostic tools and technologies for infectious and non-communicable diseases in low-and-middle-income countries. *Health Technol* 2013; 3(3).
- [12] Carl Llor, Lars Bjerrum. Antimicrobial resistance: risk associated with antibiotic overuse and initiatives to reduce the problem. *PMC* 2014; 5(6)(DOI:10.1177/2042098614554919). <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4232501/> (accessed 2 january 2018).
- [13] De WK, Allerberger F, Amann S, et al. Strategies to enhance rational use of antibiotics in hospital: a guideline by the German Society for Infectious Diseases. *PUPmed* 2016; 44(3)(DOI: 10.1007/s15010-

- 016-0885-z.).
<https://www.ncbi.nlm.nih.gov/pubmed/27066980>
 (accessed 12 march 2018).
- [14] Mahmoud.E.Ghaitas, Abduljaleel El-shalwi. *antibiotics use by inpatients at Alwahda hospital in Derna. Libya and its relationship to antibiotic resistance, a pilot study* ; 29,nov.2017.
- [15] Meredith Griffiths,Sophie Scott,. Antibiotics being incorrectly prescribed in Australian nursing homes, prompting superbug fears', *ABC*. undefined.
- [16] Oxford Biocystems. *Antimicrobial Resistance (AMR)*. <http://www.oxfordbiosystems.com/AMR> (accessed 2016).
- [17] WikiMed Medical Encyclopedia. *UK study warns of threat of antibiotics overuse, lack of new drugs'CCTV America'*.
http://iitab.me:3000/kiwix/wikipedia_en_medicine_2016-07/A/Antibiotic_therapy.html (accessed 2016).
- [18] Richard J Fair,Yitzhak Tor. Antibiotics and Bacterial Resistance in the 21st Century. *PMC* 28 Augustus 2014; 6(DOI:10.4137/PMC.S14459).
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4159373/> (accessed 13 march 2018).
- [19] Stephanie A. Pierce Hendy,Jeffery Dennis. Bacterial Culture and Antibiotic Susceptibility Testing. *compendium* July 2010; 32(7).
<https://pdfs.semanticscholar.org/3861/be8bccb4dc199507d8412ab31ef989fc64c6.pdf> (accessed February 2015).
- [20] World Health Organization. *Antimicrobial resistance Global Report on Surveillance*. Geneva - Switzerland; 2014.
http://apps.who.int/iris/bitstream/handle/10665/112642/9789241564748_eng.pdf;jsessionid=19A58EBCECC12297602A35F3DF5AB043?sequence=1 (accessed 2015).
- [21] Mary Brophy Marcus. Superbugs could kill more people than cancer, report warns', *CBSNEWS*. undefined:.
- [22] Bandla Aswani,M. Vijitha,P. Yanadaiah,Purushothama Reddy K, K.Yeswanthi,P. Vijayasanthi. Evaluation of prescribing pattern of antimicrobial agents in a multispeciality teaching hospital. *World Journal of Pharmaceutical Research* 02 June 2016 Revised on 24 June 2016; 5(8, 983-999 DOI:10.20959/wjpr20168-6757). www.wjpr.net/download/article/1469869680.pdf (accessed February 2015).
- [23] Tiemersma EW,Bronzwear SL,Lyytikainen O,Degener JE,Schrijnemakers P,Bruinsma N,et al. Methicillin resistant staphylococcus aureus in Europe 1999-2003. *Emerg Infect Dis* 2004; 10.
- [24] Jim O'Neill. *Antimicrobial Resistance: Tackling a crisis for the health and wealth of nations*. HM government : WelcomeTrust; December 2014. https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf (accessed 2015).
- [25] World Health Organization. *Global action plan on antimicrobial resistance*. Avenue Appia 20 1211 Geneva Switzerland: WHO Library Cataloguing-in-Publication Data; Avenue Appia 20 1211 Geneva Switzerland.
- http://www.wpro.who.int/entity/drug_resistance/resources/global_action_plan_eng.pdf (accessed 2015).
- [26] World Health Organization. *Global Antimicrobial Resistance Surveillance System Manual for Early Implementation*. Avenue Appia 20 1211 Geneva Switzerland : WHO Library Cataloguing-in-Publication Data; 2015.
<http://apps.who.int/iris/bitstream/handle/10665/188783/9789241549400?sequence=1> (accessed 2015).
- [27] Anna C. Seale,N. Claire Gordon,Jasmin Islam,Sharon J. Peacock,et al. AMR Surveillance in low and middle-income settings - A roadmap for participation in the Global Antimicrobial Surveillance System (GLASS). *PMC* 26 Sep 2017 ; 1(PMC5645727 :DOI 10.12688/wellcomeopenres.12527.1).
<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5645727/> (accessed Dec 2017).
- [28] Hellen Gelband,Miranda Delahoy. *Policies to Address Antibiotic Resistance in Low- and Middle-Income Countries*. https://www.cddep.org/wp-content/uploads/2017/06/abrinlmics_cddep_gelband_and_delahoy_9-14.pdf (accessed 2017).
- [29] Paul R. Rhomberg,Thomas R. Fritsche,Helio S. Sader,Ronald N. Jones. Antimicrobial susceptibility pattern comparisons among intensive care unit and general ward Gram-negative isolates from the Meropenem Yearly Susceptibility Test Information Collection Program (USA). *ELSEVIER Diagnostic Microbiology and Infectious Disease* September 2006; 56(1):.
<https://www.sciencedirect.com/science/article/pii/S0732889305003585#!> (accessed Dec 2017).
- [30] Andrea Titus. *Get Smart About Antibiotics: NewPublicHealth Q&A With Ramanan Laxminarayan*. https://www.cddep.org/blog/posts/get_smart_about_antibiotics_newpublichealth_qa_ramanan_laxminarayan/ (accessed 2017).
- [31] Student Health Center Manuals. *Kirby Bauer Antibiotic Sensitivity*. <http://shs-manual.ucsc.edu/policy/kirby-bauer-antibiotic-sensitivity> (accessed 2017).
- [32] L. Barth Reller, Melvin Weinstein, James H. Jorgensen,Mary Jane Ferraro. Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *OXFORD Academic Clinical Infectious Disease* 1 December 2009; 49(11:DOI10.1086/647952).
<https://academic.oup.com/cid/article/49/11/1749/344384> (accessed Dec 2016).
- [33] Microbe Online. *Modified Kirby-Bauer disc diffusion method for Antimicrobial Susceptibility Testing*. <https://microbeonline.com/antimicrobial-susceptibility-testing-procedure-modified-kirby-bauer-method/> (accessed 2016).

Author Profile

Mabroka Hamad Tarfaya, bachelor degree medical technology of laboratory medicine 2005, professional diploma in nosocomial infection and prevention control 2011. Work at Al-Wahda Teaching Hospital Derna Libya as head of medical laboratory and blood bank department, member of scientific committee of hospital, and head of Nosocomial Infection of Prevention control Committee

Derna-Libya Microbiology department at Al-Wahda Teaching Hospital. Study Prepares |data collection, data analysis, manuscript author, data interpretation figure design.

Mona Abdulla El-mahdi, bachelor degree medical technology of laboratory medicine 2001, work at Al-Wahda Teaching Hospital Derna Libya as laboratory specialist and microbiologist until 2017. Study prepares |samples preparation and work load and processes of data collection

Zahra Mohamed Abomusa, bachelor degree medical technology of laboratory medicine 1999, work at Al-Wahda Teaching Hospital Derna Libya as laboratory specialist and microbiologist. Study prepares |samples preparation and data collection