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Research article

CLINICAL ISOLATES OF *MECA*, METHICILLIN, VANCOMYCIN RESISTANCE *S. AUREUS*; ESBLs PRODUCING *K.PNEUMONIA*, *E.COLI*, *P. AUREGENOSA* FROM VARIOUS CLINICAL SOURCE AND ITS ANTIMICROBIAL RESISTANCE PATTERNS

*Ismail Mahmud Ali¹, Amirthalingam R²

¹Hospital Director, Head, Assistant Professor, Department of Surgery, Ibn Sina Teaching Hospital,

²Specialist, Dept of Molecular biology, Ibn Sina Teaching Hospital, Sirt University, Libya, P.O. Box 705

*Corresponding author email: amrithrgenes@yahoo.co.in

ABSTRACT

Background and Objective: Antimicrobial resistance has turned into a key medical and public health crisis globally since the injudicious use of magic bullets (drugs). Aim of this study is focused on the clinical isolate and their percentages of resistant to antibiotics in gram positive bacteria such as MRSA, VRSA, and MSSA are common causes of nosocomial, skin structure infections, bacteremia and infection of other systems; ESBLs producing *Enterobacteriaceae* (*E. coli*, *Klebsiella spp.*) is common agent of urinary tract, bloodstream, pulmonary and intra-abdominal infections and carbapenem resistant *P. aeruginosa* with its complete antimicrobial patterns which are currently practiced in this population. **Methods:** There are one hundred and fourteen (114) various clinical isolates, isolated from various clinical samples like throat swab, urine, pus, sputum, and blood culture, identified as specific isolate with resistance patterns were analyzed by BD phoenix-100 the auto analyzer. **Results:** Off 114 clinical isolate, 6 *mecA*-mediated resistance (cefoxitin > 8 mgc/ml), 11 methicillin resistance, 18 lactam/ lactamase inhibitor, 12 methicillin sensitive and 3 vancomycin (>16 µg/ml) resistance *S. aureus* have been isolated from overall 50 isolate of *S.aureus*. In addition, there are 27 *P.aeruginosa*, 15 ESBLs from overall of 25 *K. pneumoniae* and 7 ESBLs out of 12 *Escherichia coli* species have been isolated. The resistance and susceptibility pattern percentages have been graphically represented for each isolates. **Conclusion:** Current study revealed that the drug classes of lactam/ lactamase inhibitor having high resistance rate with *S.aureus*, *P.aureginosa*, *K. pneumoniae* and *E. coli* isolate. Also, some of other drug classes such as cepham and tetracycline having higher resistance rate with *P.aureginosa* and *K.pneumoniae*. In addition, the vancomycin resistances *S. aureus* have been isolated and reported as first time in this population.

Keywords: Methicillin & Vancomycin Resistance, Methicillin Sensitive *Staphylococcus aureus*; extended spectrum lactamases (ESBLs).

INTRODUCTION

A number of bacterial infections are caused by gram negative aerobic and facultative anaerobic bacteria, it belongs to the family of *Enterobacteriaceae* and *non-Enterobacteriaceae*. The gram positive bacteria belong to the genera *Staphylococcus*, *Enterococcus*, gram positive cocci and gram positive bacilli, all of them are causative agents of nosocomial and

community acquired infectious diseases in human habitual life. Nearly 10 million children under the age of 5 years have been died until the period of 2008 and this effect enhanced the socioeconomic condition in many developing countries, better management in infection control, fast access immunization and introduction of new vaccines against bacterial

infection diseases¹⁻². The aerobic and facultative anaerobic gram negative bacterium such as *K. pneumoniae*, *E. coli* and *P. aeruginosa*, and gram positive bacteria like *S. aureus* are multi-drugs resistance bacterium and currently it is very common in this local population as well as Ibn Sina Teaching Hospital setup. This antimicrobial resistance is a natural biological phenomenon and it is operated by a genetic material (Nucleic acid) of living microbes such as plasmids, transposons and integrons, it has major roles in extension of multi-drugs resistance in all bacterium³. *Staphylococcus aureus* is an opportunistic hospital associated (HA-MRSA) and community associated (CA-MSSA) infectious bacterium in human body. The one called methicillin resistant *Staphylococcus aureus* (MRSA) contain a *mecA* gene that makes resistance to all -lactam antibiotics including penicillin and cephalosporin; methicillin sensitive *Staphylococcus aureus* (MSSA) susceptible to many antibiotics other than lactam, and vancomycin resistance *S. aureus* (VRSA) resistant to long time use of glycopeptides antibiotics. It can cause soft tissue infection as well as pneumonia, endocarditic, septic arthritis, osteomyelitis, meningitis and septicemia. Hence, Hospital acquired MRSA isolates are major causes of nosocomial infections. The community associated MRSA strains are significantly associated with soft tissue infection, it also causes sepsis, necrotizing fasciitis, and necrotizing pneumonia. It is an epidemic as well as endemic all over the world⁴. Extended spectrum lactamases (ESBLs) are enzymes produced by different types of *Enterobacteriaceae* family including *Klebsiella pneumoniae*, *E. coli*, and non-fermentative gram negative bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. It is derived from (Temoniera patients) TEM or sulphhydroxyl variable (SHV) enzymes (for resistance of ceftazidime and ceftriaxone antibiotic) types of plasmid mediated lactamases through gene mutation (amino acid alteration). The CTX-M type enzymes are replacing SHV and TEM type's enzymes in nosocomial and community acquired infections caused by *E. coli* and *K. pneumoniae*⁵. The *K. pneumoniae carbapenemase* (KPC) is an enzyme of *K. pneumoniae* for resisting multi-drugs like lactams, fluoroquinolones, and amino glycosides drug classes. These types of KPC infection may cause high therapeutic failure with 50% of mortality rates⁶⁻⁷.

Current study mainly focused on the isolation of resistant and multi-drugs resistant pathogenic bacterium and its antimicrobial susceptibility patterns. The ultimate goal of this study is to guide the health care workers and specialists to manage such type of infections in an ethical and scientific way and also guide for the society of infectious disease panel and Ministry of Health in Libya.

MATERIALS AND METHODS

Clinical Samples: In the prospective analysis, a total of 114 different bacterial isolates have been identified from different clinical sources. The clinical sources were sputum, urine, pus, wound & throat swabs, and blood culture, received from Ibn Sina Teaching Hospital and Out Patients during the years of 2013-2014. Although, some of exist data's during the years of 2008 were integrated in this research for highlighting the vancomycin resistance *S.aureus* have been isolated previously by BD expert in this locality. The dose of each drugs have been mentioned in all graphs, because of the drugs concentration were differed in each pathogenic bacterium so please refer to the graphs. The drugs classes were included for all clinical isolate such as 5-fluoroquinolone (Ciprofloxacin-CIP, Norfloxacin-NOR, Levofloxacin-LUX, Moxifloxacin-MXF) ; Amino glycoside (Gentamicin-GM, Amikacin-AN); lactam/beta-lactamase Inhibitor (Ampicillin -AM, Penicillin-G,-P,Oxacillin-OX, Amoxicillin/Clavulanate-AMC, Piperacillin-PIP); Carbapenam (Imipenem-IPM, Meropenem-MEM); Folate Antagonist (Trimethoprim/ Sulfamethoxazole-SXT); Glycopeptides (Vancomycin-VA); Cephem (Cephalothin-CF,Cefuroxime-CXM,Cefoxitin-FOX,Ceftazidime-CAZ,Cefotaxime-CTX, Cefepime-CEP, Ceftriaxone-CRO); Lincosamide (CC-Clindamycin); Macrolide (E-Erythromycin); Nitro(FM-Nitrofurantion) ; Oxazolidine (LZD-Linezolid) and Tetracycline. These are drugs names with drugs classes have been applied according to the gram stains concerns and rest of drugs classes have been considered as exclusion criteria. The ethics panel and internal review board of the organization approved the procedure.

Culture Media preparation: There are some routine mediums such as mac-conkey agar, nutrient agar, blood agar (rest of clinical source and sensitivity), chocolate agar (CSF), CLED agar for urine sample

(Cystine-Lactose-Electrolyte-Deficient) and blood culture bottle Bactech for blood samples (aerobic & an-aerobic) were been used in the department of clinical microbiology laboratory. These are all powdered form except blood culture bottle and it has been diluted according to the manufacturers guidelines in the individual conical flask and further autoclaved at 125 psi for 30 minutes, then it has been cooled to room temperature and dispensed the liquid substance with slightly lukewarm condition on culture plates. Day by day the samples were received; cultured, isolated, gram stained and applied as diluted samples to the auto-microbial analyzer for further confirmatory reasons. Hence, the culture medias are recommended by manufacture to use in ID and AST (antimicrobial susceptibility test) broth in system analysis such as trypticase soy agar with 5% sheep blood, bromthymol blue (BTB) lactose agar, BBL™ chromagar™ orientation; chocolate agar; columbia agar with 5% horse blood; columbia agar with 5% sheep blood; cystine-lactose-electrolyte-deficient agar (CLED); dey/engley (D/E) neutralizing agar; eosin methylene blue; hektoen enteric agar; macconkey agar; trypticase soy agar without blood; trypticase soy agar with lecithin and tween 80 and xylose lysine desoxycholate agar. Hence, dey/engley (D/E), neutralizing agar; hektoen enteric agar; trypticase soy agar without blood; trypticase soy agar with lecithin and tween 80 and xylose lysine desoxycholate agar are not recommended to use for AST broth in the system analysis.

Bacterial Culture Isolation: The Major clinical isolate have been confirmed with gram stains to get verification of weather gram positive or negative bacterium. After that, the clinical isolate have been specified the bacteria, biochemical tests, and its multi-drug susceptibility tests were performed through BD-Phoenix-100, the microbial auto analyzer.

BD Phoenix Panel: There are three more BD phoenix panel kits have been introduced by manufacturer to screen bacterial pathogens and each panel has its own specific pathogenic identification with drugs sensitivity and resistance patterns. Such as PMIC/ID-69, PMIC and PID panels (Gram positive pathogenic), NMIC/ID-94, NMIC and NID panels (Gram negative pathogenic organisms) and special panels only for SMIC/ID-11 panels (Streptococcus species). The system can perform at least 100 bacterial

identification with antimicrobial susceptibility at a time. It utilizes an optimized colorimetric redox indicator for AST, and a diversity of colorimetric and fluorometric indicators for ID. The AST broth is cation (Ca^{++} and Mg^{++}) adjusted to optimize susceptibility testing performance⁸.

Bacterial Identification: The ID portion of the Phoenix panel utilizes a series of conventional, chromogenic, and fluorogenic biochemical tests to determine the identification of the organisms. The ID broth contains 45 wells with dried biochemical's substrate and two fluorescent control wells. Both growth-based and enzymatic substrates are employed to cover the different types of reactivity in the range of taxa. The tests are based on microbial utilization and degradation of specific substrates detected by various indicator systems. Acid production is indicated by a change in the phenol red indicator when an isolate is able to utilize a carbohydrate substrate. The chromogenic substrates produce a yellow color upon enzymatic hydrolysis of either p-nitro phenyl or p-nitroanilide compounds. Enzymatic hydrolysis of fluorogenic substrates results in the release of a fluorescent coumarin derivative. Organisms that utilize a specific carbon source reduce the resazurin-based indicator. In addition, there are other tests that detect the ability of an organism to hydrolyze, degrade, reduce, or otherwise utilize a substrate⁹⁻¹⁰.

Antimicrobial Susceptibility Testing: The BD Phoenix AST method is a broth based micro dilution test. It contains 84 wells with dried antimicrobial agents and one growth control well. The Phoenix system utilizes a redox indicator for the detection of organisms' growth in the presence of an antimicrobial agent. Continuous quantification of changes to the indicator as well as bacterial turbidity is used in the determination of bacterial growth. Each AST panel configuration contains several antimicrobial agents with a range of two fold doubling dilution concentrations. Organism identification is used in the interpretation of (the lowest concentration of an antimicrobial agents in which no visible growth occur is called minimal inhibitory concentration) MIC values of each antimicrobial agent producing Susceptible, Intermediate, or Resistant (SIR) result classifications¹¹.

BD Phoenix-100 Methods: BD Phoenix is an automated microbiology system utilized for

recognition of pathogenic bacteria from pure culture of different clinical samples. The pathogenic organisms include the species of *Staphylococcus*, *Enterococcus*, Gram positive bacilli and cocci, Gram negative aerobic and facultative anaerobic bacteria the family of *Enterobacteriaceae* and *non-Enterobacteriaceae*. Also, it is future for in vitro quantitative determination of antimicrobial susceptibility by minimal inhibitory concentration (MIC).

The pure culture isolates have been tested with a gram stain to assure the appropriate selection of Phoenix panel type. Using aseptic technique, bacterial colonies were picked from culture plate by sterile swab and suspend the colonies into 4.5 ml of ID broth. The BD Phoenix Spec Nephelometer has been used to make inoculums densities purposes. There are two types of inoculums density have been applied (0.25 & 0.5 McFarland) for the panel type being run, and then it ranges of 0.20 -0.30 to 0.50-0.60 was acceptable. If the density of organisms is low then it needs addition of bacterial colonies. Later on one drop of AST indicator solutions was added to AST broth solution (8.0ml); then 25µl or 50µl of bacterial suspension were transferred from the ID tube into AST broth tube according to the density of suspension. These panels have been loaded into the Phoenix instrument and continuously incubated at 35°C until 16 hrs for the results gaining. There are three different antibiotic methods (PMIC, NMIC and SMIC) were been utilized in BD Phoenix-100 system and the results were been reported as 95% confidence automatically according to the biochemical reaction the report have been released with pathogenic names. Statistical analysis was done by using micro software excel 2007.

RESULTS

There are 50 different types of *S. aureus* have been isolated from various clinical samples with different drug resistance patterns. Among fifty isolate, 6 *mecA* mediated resistant *staphylococcus* (MIC=cefoxitin>8mcg/ml), 11 methicillin (resistance of ampicillin, penicillin, oxacillin and amoxicillin/clavulanate), 18 lactamase producing *staphylococcus aureus* (susceptible to oxacillin and amoxicillin), 12 methicillin sensitive (ampicillin, oxacillin and amoxicillin/clavulanate) 3 vancomycin (>16µg/ml) resistance *S.aureus* and some of

heterogeneous vancomycin intermediate isolate have been differentiated through auto analyzer from various clinical samples. The selective antibiotic usages have been graphically explained as percentages with susceptible and resistance patterns for *S.aureus* (refer to Fig-1). The most drug resistance such as ampicillin (94%), penicillin (100%), oxacillin (70%) and amoxicillin/clavulanate have been observed as high resistance ratio in among isolate of *S. aureus*. The methicillin resistances *S.aureus* are reported as high resistance to penicillin, lactam/ -lactamase inhibitor combinations, cephalosporin's and carbapenem because of ineffectiveness in clinical usage but in this study carbapenem (imipenem 56% and meropenem 66%) is susceptible for *S.aureus*.

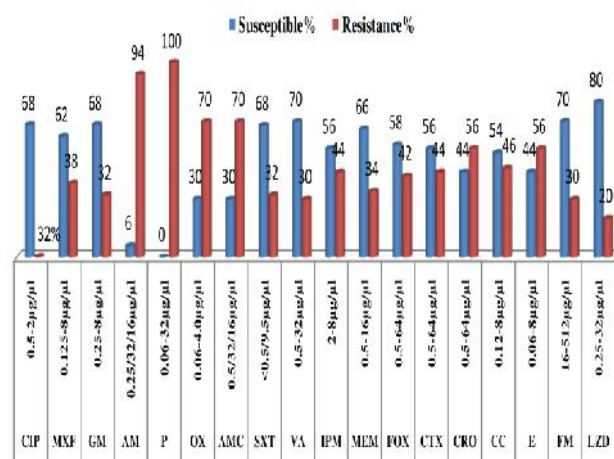


Fig 1: Antimicrobial resistance patterns for *S.aureus*

Non-fermentative isolate such as *Pseudomonas aeruginosa* is most frequently isolated as multidrug resistance gram negative bacilli in routine clinical microbiology laboratory. In current analysis, there are twenty seven selective isolate have been isolated from different clinical sources. The multidrug resistance patterns have been observed as high in lactam/ lactamase inhibitor (96-100%), folate antagonist (100%), cephem (96-100%), and tetracycline (100%). Hence, the moderate resistance patterns also observed in 5-fluoroquinolone, amino glycoside and carbapenem (Fig-2). Generally, *P.aeruginosa* is intrinsically resistant to tetracycline; primary quinolones, first generation and second generation cephalosporin, amino penicillin/beta-lactamase inhibitor combinations, ertapenem, ceftizoxime, cefotaxime, ceftriaxone, chloramphenicol, trimethoprim, trimethoprim/sulfamethoxazole and kanamycin were been

mentioned by BD expert. *P.aeruginosa* may develop resistance during prolonged antimicrobial therapy with all antibiotics. Norfloxacin and Tetracycline are drugs of choice for urinary tract infection.

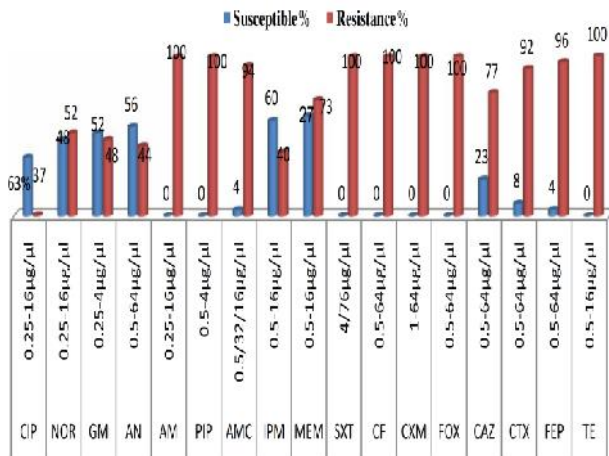


Fig 2: Multidrug resistance patterns for *P.aeruginosa*

Enterobacteriaceae family, mainly *Klebsiella pneumoniae* and *Escherichia coli* are producing lactam/ lactamase enzymes are able to deactivate the antibiotic like lactam/lactamase drugs classes and it also found in *Pseudomonas aeruginosa*. The current analysis revealed that, more than 25 isolates from different clinical samples such as sputum, urine, pus, and throat swab. The resistance patterns of *Klebsiella pneumoniae* have been graphically explained (Fig-3).The highest resistance patterns were observed in lactam/ lactamase and 5-fluoroquinolone drug classes. The highest susceptibility prevalence was observed in the drug class of carbapenem (88%).

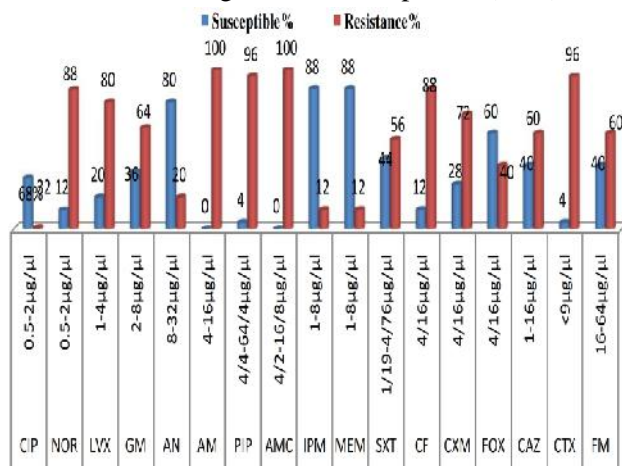


Fig 3: Multidrug resistance patterns for *K.pneumoniae sp*

Finally, the extended spectrum lactamase producing *E. coli* isolate were observed among twelve isolates. The highest drug resistance patterns were observed in lactamase and tetracycline drugs classes and

susceptibility rate was high in carbapenem than other drug classes (Fig-4).

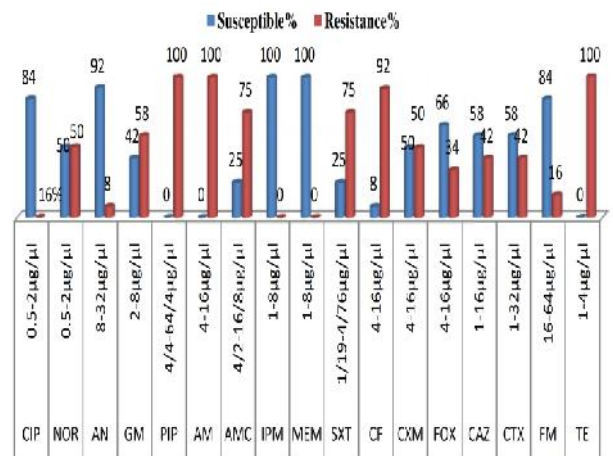


Fig 4: Antimicrobial resistance patterns for *E.coli*

DISCUSSION

Resistance to antimicrobial drugs is a major health crisis in Libya like others developing nations because of lack of antimicrobial resistance survey and improper management of drugs. Generally, *Staphylococcus aureus* is well recognized as causative agent of hospital-and community acquired infections in this locality and it was first isolated as MRSA in Benghazi in the year of 1972 by Goda¹². Later on it was reported in different Cities^{13, 14, 15}. Also, vancomycin resistance (MIC 16µg/mL), intermediate (4-8µg/mL) and heterogeneous (1-4µg/mL) *S. aureus* have been reported in US, Europe, and Asia¹⁶⁻¹⁷. However, there is no VRSA, VISA and hVISA cases were reported in Libya by using standard CLSI methods until 2011. Whereas in present study, MRSA, MSSA, ESBLs producer and vancomycin resistance *S. aureus* (MIC 16µg/mL) have been isolated and in brief high lightened with resistance patterns in results division. Hence, some of isolates were found with vancomycin sensitive, heterogeneous and intermediate *S. aureus*.

Enterobacteriaceae spp mainly *Klebsiella pneumoniae* and *E. coli* bacterium are gram negative bacterium. The *K. pneumoniae* OXA-48 producing strains have been reported mainly from North African countries, the Middle East, Turkey, India and other European countries¹⁸⁻¹⁹. This bacterium steadily in advance to resistance against -lactam antibiotics like penicillin and cephalosporin by the assembling of extended-spectrum lactamases (ESBLs) or carbapenem-hydrolyzing enzymes for *K. pneumoniae*²⁰. The another mobile metallo -lactamases enzymes

produced by *Pseudomonas aeruginosa* spp in the course of multidrug resistance activity and this type of enzymes synthesis could be assorted from nation to nation²¹. Previous study of *E.coli* drugs resistance pattern have been reported from Sirt, Tripoli and Benghazi, during the years of 2002-2008. The resistance pattern were ampicillin (49%,59%,57%); chloramphenicol (23%,21%,14%); gentamicin (9%,10%,7%); nalidixic acid (28%,28%,23%); ciprofloxacin (2%,14%,17%) and trimethoprim-sulfamethoxazole (36%,24%, 31%).^{22,23,24}. Similarly, the *K. Pneumonia* isolates proved to be resistant to all broad-spectrum cephalosporin and to fluoroquinolones and susceptible to tigecycline were reported from Italy during the Libyan conflict²⁵. Regarding resistance pattern in present study (*K.pneumonia* & *E.coli*) were differed slightly from previous report and should target more scientific research on carbapenem and metallo -lactamases producing isolates by molecular analysis in this locality in future.

The antibiotic resistance pattern in developing countries like Iran, nearly 33% of ESBLs producing *K. pneumoniae* causing major curative troubles and it resistant was to ciprofloxacin and amino glycosides²⁶; though in India ESBL isolate drugs resistance were amoxicillin (93.3%), gentamicin (70%), ciprofloxacin (10.4%) and amikacin (26.1%)²⁷. Likewise in present study, lactam/lactamase drug class having high resistance followed by cepham drug class for *K.pneumoniae* and susceptibility ranges was higher in carbapenem drugs class. But in *E .coli*, lactam/ lactamase drugs class having high resistance followed by tetracycline drug class and its susceptibility pattern were 5- fluoroquinolone aminoglycoside and carbapenem drugs class than nitrofurantoin and cephem drugs class. The superior antimicrobial resistance rates in current study could be attributed to the resource of the clinical isolates being from multispecialty setup and recurrent usage of broad spectrum antibiotic. Also study might be helpful to control of infectious diseases in public as well as poor setup of hospitality.

CONCLUSION

Present study highlighted that the drug classes of lactam/ lactamase inhibitor having a high resistance rate with *S. aureus*, *P.aureginosa*, *K. pneumoniae* and *E. coli* isolate. Hence, some of other drugs classes

such as cepham and tetracycline also having higher resistance rate with *P.aureginosa* and *K.pneumoniae*. In addition, the vancomycin resistances *S. aureus* have been isolated first time in this region by BD phoenix expert and the clinical specialists should concentrate much more in these cases. As a result, superior antibiotic policy and hospital infection management assessment can be making the first move to prevent the emergence of multidrug resistant clinical isolates.

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Conflict of interest: None

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