WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 12-22. Research Article ISSN 2277– 7105

ISOLATION AND IDENTIFICATION OF MULTIDRUG RESISTANCE PATHOGENIC BACTERIAL FROM SKIN INFECTIONS

Arwa M. Salih*, Mayada S. Mahdy and Hameed M. Jasim

Collage of Biotechnology, Al-Nahrain University, Baghdad- Iraq.

Article Received on
25 Sept. 2018,
Revised on 15 Oct. 2018,
Accepted on 04 Nov. 2018
DOI: 10.20959/wjpr201819-13675

***Corresponding Author**
Dr. Arwa M. Salih
Collage of Biotechnology,
Al-Nahrain University,
Baghdad- Iraq.

ABSTRACT

A total of (40) samples were collected from patients suffering from different skin infections at different ages and from both genders in (2) different hospitals in Baghdad during the period from August/2017 to October/2017. Bacterial isolates were identified by a microscopic examination and cultural characteristics on different selective media MacConkey agar, EMB agar, Manitol salt agar, Skim milk agar, Blood agar, King A and King B agar. A further identification by Vitek system revealed that only 69 isolates gave typical morphological characteristics and biochemical tests related to *Enterobacter cloacae* 12 (24%), *Klebsiella Pneumonia* 1 (2%), *Proteus mirabilis* 1 (2%), *Pseudomonas aeroginosa* 15 (30%) *and Staphylococcus aureus* 21 (42%). The rest isolates belonged to pathogenic bacteria from different genera like *S.epidermidas* 11 (15.9%), *Bacillus* spp 6 (8.6%) and *Pseudomonas stutzeri* 1(1.4%). An antibiotic sensitivity test was used for the detection of the susceptibility of different skin infections isolates against (9) antibiotics. According to antibiotic susceptibility tests, eighteen bacterial isolates were selected, which are found to be resistance to Gentamycin, Cefotaxime, Amikacin, Ceftriaxon, Tobramycin and Amoxiclar.

KEYWORD: *Enterobacter cloacae, Klebsiella Pneumonia, Proteus mirabilis.*

INTRODUCTION

Skin is one of the largest organs in the human body in terms of size and weight. The average adult skin surface area is 1.5 to 2.0 square meters. An intact human skin surface is vital to the preservation of body fluid homeostasis, thermoregulation, and the host's protection against infection. The skin also has immunological, neurosensory, and metabolic functions such as vitamin D metabolism (Church *et al.*, 2006). More than 200 different species of bacteria

normally live on the skin (Benbow, 2010), and an open wound provides a moist, warm and nutritious environment perfect for microbial colonization and proliferation (Young, 2012). When one or more microorganisms multiply in the skin, local systemic responses occur in the host, which can lead to infection and a subsequent delay in healing (Angel *et al.*, 2011). It was found that when chronic skin infections are poorly perfused they are more susceptible to infection, as blood delivers oxygen, immune cells and nutrients, thus providing little opportunity for microorganisms to colonize and proliferate (Bowler *et al.*, 2001). Skin infections are a major focus for infection, as they become readily colonize with several species of potentially pathogenic microorganisms, including *Pseudomonas aeruginosa* and *Staphylococcus* sp. (Mayhall, 2003). The increasing frequency of multidrug-resistant strains is concerning, as efficacious antimicrobial options are severely limited. Risk factors for infection include prolonged hospitalization, exposure to antimicrobial therapy, and immunocompromised states such as human immunodeficiency virus infection (Obritsch *et al.*, 2005).

MATERIALS AND METHODS

Test microorganism

Pathogenic bacteria (*Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus).*

Samples collection and cultivation

Specimens were collected from 69 patients of different ages and genders suffering from skin infections attends to Al-Yarmook Teaching Hospital from August to July/2017 in Baghdad governorate. Specimens were taken from different skin infections by using sterile disposable cotton swabs and kept into test tubes containing transport medium (Stuart transport medium). After transferring to College of Biotechnology labs, 100 µl aliquots were taken from each test tube and used to inoculate Lauria Bertani broth medium and incubated at 37˚C and 55˚C for 24 hrs., until the presence of heavy growth.

Sample processing and bacterial identification

All the clinical samples were incubates onto blood agar, MacConkey agar, mannitol salt agar, King A, King B, skim milk agar. All the culture plates were incubated at 37˚C for overnight. The plates showing growth of bacterial were processed for identification (*Enterobacter cloacae, Klebsiella pneumonia, Proteus mirabilis, Pseudomonas aeruginosa* and *Staphylococcus aureus)* using stander microbiology procedures and performing specific

biochemical tests catalase test, Indole test, oxidase test, urease test and citrate test. To ensure identification of the bacterial species, VITEK 2 system was used through its Gram-negative and Gram-positive cards. By this system of identification, 37 bacterial isolates were found to be belonging to five different species.

Antibiotic Susceptibility Test

Antibiotic susceptibility test was performed by using Kirby Bauer's disc diffusion method according to the manual on antimicrobial suspectibility testing (2004). Results were compared with Clinical Laboratory Standards Institute (CLSI) (2016). Inoculum of bacterial isolates was prepared by suspending fresh culture of each bacterial isolate in saline solution to match 0.5 McFarland turbidity standard.

A sterile cotton swab was dipped into the bacterial suspension and streaked on Muller-Hinton agar plates then plates were left at room temperature for 3 to 5 min to allow adsorption of excess moisture. After that, antibiotic discs were placed on the surface of inoculated plates and incubated at 37◦C for 18-24 hrs. After incubation, diameters of the inhibition zones were measured in mm and compared with standards of CLSI. Antibiotic disc indicated in table (1) were supplied by bioanalyse/Turkey

Table (1): Antibiotic discs used in this study.

Antibiotics	Symbol	Concentration (µg/disc)
Amikacin	AK	10
Amoxiclar	APC	10
Ceftriaxon	CRO	10
Cefotaxime	CTX	30
Ciprofloxacin	CIP	10
Gentamycin	GN	10
Levofloxacin	LEV	5
Tobramycin	TOB	10
Trimethoprim	TMP	10

RESULTS AND DISCUSSION

Collection of samples from skin infections

A total of 40 samples were collected from patients suffering from different skin infections, attends Al-Yarmouk hospital Teaching and AL-Imamein AL-Kadhimein medical city in Baghdad governorate during the period from August /2017 to October /2017. Swab samples were spread on Lauria Bertani agar medium and incubated at 37 ˚C and 55 ˚C for 24 hrs to

allow the growth of bacterial colonies. From 40 samples, only 37 isolates gave bacterial growth, where a total of 69 different isolates were obtained at these two temperature of incubation. These isolate were further purified and subjected to full identification according to their morphological and cultural characteristics and biochemical tests.

Identification of bacterial isolates

The forty bacterial swab samples obtained from different clinical samples were identified primarily to the genus according to microscopic examinations and cultural characteristics, and then the to species according to the results of biochemical tests. Final identification was confirmed by using VITEK 2 system.

Microscopic characterization

Results showed that 15 (30%) of the total isolates were Gram negative, straight or slightly curved rods which suspected to belong to *Pseudomonas* spp, 1 (2%) of the isolates were Gram-negative, rod-shaped and appears in pairs or short chains under light microscope. Isolates with such characteristics were suspected to be belong to *Klebsiella* ssp. 12 (24%) of total isolates were appeared as coccobacilli, or straight rods with rounded ends which suspected to belong to be *Enterobacter* (Forbes *et al.*, 2007). 1(2%) of the isolates were, gram negative short rods, such characteristic which suspected to be belong to *Proteus ssp.* (Brooks *et al.*, 2007), and finally 21 (42%) of the total isolates were cocci arranged in irregular clusters, uniformly positive for gram stain reaction, non-motile and nonspore forming were suspected to be belong to Staphylococci AL-Kazaz (2006). Other isolates obtained in this study 11 (15.9%) *S.epidermidas*, 6 (8.6%) *Bacillus* spp., 1(1.4%) *Pseudomonas stutzeri*.

Cultural characterization

Identification of the 69 bacterial isolates were performed at first depending on the characteristics of colonies grown MacConkey agar medium and blood agar medium. Furthermore, many bacterial isolates were allowed to grow on the selective media that are more specific for their species as EMB agar, mannitol salt agar, skim milk agar, King A and King B agar medium. Regardless to lactose fermenting ability, bacterial isolates were able to grow on MacConkey agar medium with various shapes and morphologies were belong to, *Pseudomonas* ssp., that they were propagated on MacConkey agar, were non-lactose fermentor and their colonies were appeared small (1- 3 mm in diameter) circular, entire, pale converting media color from red to pink (Sousa *et al*, 2013). On blood agar, these bacterial isolates display beta hemolysis, a metallic sheen, and blue or green pigment and grape-like

odor (Garrity *et al.*, 2005). On the other hand, subculturing these isolates on skim milk agar medium, they were gave grape-like smelling with bluish- green color which refer to pyocyanin production (Ravel and Cornelis, 2003). *P. aeruginosa* is known to produce two types of pigments, pyocyanin and fluorescein which is a characteristic property and aids in isolation of *Pseudomonas* from clinical material. King A base medium is particularly suited for the production of pyocyanin and pyorubin, this medium can be used as a general medium for the non-selective isolation and pigment production of *Pseudomonas* ssp., these media contain proteose peptone, which provides carbonaceous and nitrogenous compounds for the growth of bacteria, glycerol serves as a source of energy and also enhances pigment production, magnesium chloride, potassium sulphate and magnesium sulphate also enhances pigment production (Murray *et al.*, 2003). King B base medium contain proteose peptone, which provides carbonaceous and nitrogenous compounds for the growth of bacteria. Glycerol serves as a sole source for carbon and energy, and also enhances pigment production. Magnesium sulphate also enhances pigment production. Pigments and their derivatives produced by *Pseudomonas* ssp. play a vital role as Siderophores in the iron uptake systems of bacteria, and hence, their production is markedly enhanced under conditions of iron deficiency. The production of pigments especially non-fluorescent blue pigment, pyocyanin is readily demonstrated by culturing on Kings Medium B, which contains no added iron (Todar, 2006). Addition of dipotassium phosphate to the medium increases the phosphorus content thereby enhancing production of fluorescent pigment (Murray *et al.*, 2003).

Other isolates were found to be lactose fermentor, dome- shaped colonies, after overnight incubation at 37 ˚C with a mucoid aspect and sometimes stickiness which they may be suspected to be *Klebsiella* ssp., they were appeared grey, round, shiny, non-hemolytic and mucoid colonies on blood agar medium (Garrity *et al.*, 2005). Bacterial isolates suspected to be *Klebsiella* ssp. were subcultured on Eosin Methylene Blue (EMB) agar medium. Results showed that colonies of these isolates are purple dark centered mucoid due to lactose fermentation and acid production (Leboffe and Pierce, 2011).

On the other hand other bacterial isolates were belong to *Proteus* ssp as they where opaque grayish colonies on blood agar medium with a rapid spreading appearance (Garrity *et al.*, 2005),while bacterial isolates appeared with dark pink color or pale on MacConkey agar

medium were suspected belong to *Enterobacter* spp with purple dark centered due to lactose fermentation and acid production (Leboffe and Pierce, 2011).

Staphylococcus isolates were large, creamy white and form beta hemolytic colonies on blood agar medium. On the other hand, isolates formed non-hemolytic creamy white colonies on mannitol salt agar medium which suspected to belong to Coagulase Negative *Staphylococci* (Gillespie and Hawkey, 2006). Furthermore, isolates belong to *Staphylococcus* ssp were appeared as subculture on mannitol salt agar medium which considered selective and differential medium for this genus. Results also showed that these isolates had the ability to ferment mannitol sugar and form large golden colonies surrounded by wide yellow zones and turned the color of the medium from pink to yellow, the development of yellow halos around the bacterial growth is presumptive evidence that the organism is a pathogenic *Staphylococcus* (usually *S. aureus*). Other isolates were mannitol non fermentor which appeared as small pink colonies belong to *S. epidermidis* (Leboffe and Pierce, 2011).

Biochemical characterization

Bacterial isolates identified as *Pseudomonas* ssp., *Klebsiella* ssp., *Proteus* ssp., *Enterobacter* ssp., and *Staphylococcus* ssp, according the results of their cultural and morphological characteristics were further identified according to their biochemical reactions. Results indicated in Table (2) showed that 12 (24%) were primarily identified as *E. cloacae* because they are negative for indole and urease tests, while they are positive for oxidase and citrate utilization tests. One isolate (2%) was identified as *K. pneumonia* because it was negative for indole and oxidase test while it was positive for citrate utilization tests. Five isolates (30%) were identified as *P. aeroginosa* as they were negative for indole and urease tests, while positive for oxidase and citrate utilization tests. One isolate (2%) was identified as *P.mirabilis* because it was negative for indole test, while positive for oxidase, citrate and urease utilization tests. Four (42%) were identified as *S. aureus* as they were negative for oxidase, while positive for indole, citrate and urease utilization tests.According to these results of biochemical tests, which were agree with (Holt *et al.*,1994; Garrity *et al.*,2005).

Table (2): Biochemical tests for identification of bacterial isolates obtained from skin infection patients.

Biochemical test	Bacterial isolate				
	E.cloacae	***K.pneumonia***	***P.aeroginsa***	***P.mirabilis***	***S.aureus***
Shape	Rod	Rod	Rod	Rod	Cocci
Gram staining	_	_	_	_	+
Indole	_	_	_	_	+
Oxidase	+	_	+	+	_
Citrate utilization	+	+	+	+	+
Urease	_	+	_	+	+

(-):negative result; (+): positive results.

Antibiotic susceptibility of pathogenic bacterial

Investigation of antibiotic susceptibility pattern of bacteria is a useful method for determining future challenges of effective therapy. Disc diffusion method was used in this study to investigate antibiotic susceptibility of isolates from different skin infections was examined, 69 bacterial isolates pathogenic isolates were found to exhibit different obvious levels of resistance and susceptibility pattern for nine different antibiotics. The diameter of zone of inhibition for these isolates compared to the CLSI was illustrated in Table (3).

Table 3: Resistance percentage of bacterial isolates isolated from different skin infections to different antibiotic.

Bacterial Isolates	GN	TMP	CTX	AK	CIP	TOB	CRO	APC	LEV
E. cloacae	8.6%	4.3%	7.2%	8.6%	0%	10%	10%	10%	0%
K. pneumoniae	1.4%	0%	0%	1.4%	0%	1.4%	1.4%	1.4%	0%
P. mirabilis	1.4%	0%	0%	0%	0%	1.4%	1.4%	1.4%	0%
P.aeruginosa	7.2%	7.2%	7.2%	0.02%	0%	7.2%	7.2%	7.2%	0%
S. aureus	5.7%	5.7%	0.02%	1.4%	0%	5.7%	5.7%	5.7%	0%

GN: Gentamycin; TMP: Trimethoprim; CTX: Cefotaxime; AK: Amikacin; CIP: Ciprofloxacin; TOB: Tobramycin; CRO: Ceftriaxon; APC: Amoxiclar; LEV: Levoflaxacin

According to antibiotic susceptibility tests, eighteen bacterial isolates were selected, which are found to be resistance to Gentamycin, Cefotaxime, Amikacin, Ceftriaxon, Tobramycin and Amoxiclar. Multidrug resistant bacterial infections are spreading worldwide where Extended Spectrum β-Lactamases (ESBLs) are the major MDR (multidrug resistant) related bacterial enzymes in addition to AmpC β-lactamases (Chakraborty *et al.*, 2011). The increasing ability to make altered receptors for antimicrobial agents, enzymes to destroy antibiotics and resistant metabolic pathways have significantly increased drug resistances in

Gram negative pathogens (Parajuli *et al.*, 2017). Antimicrobial resistance (AMR) is defined as resistance of a microorganism to an antimicrobial medicine to which it was originally sensitive. AMR is a natural phenomenon, which is amplified by continuous and unnecessary exposure to antimicrobials (WHO 2016). According to European Center for Disease Prevention and Control (ECDC), for the Gram negative bacteria such as, *Pseudomonas aeruginosa, Klebsiella pneumonia, Enterobacter* ssp and *proteus* ssp, multidrug resistance is defined as non-susceptible to at least one agent in at least three different antimicrobial categories. The antimicrobial categories are exclusive for the different organisms (Magiorakos *et al.,* 2012). ESBLs represent a major group of β-lactamases which have the ability to hydrolyze and cause resistant to various type of newer β-lactam but not the cefotaaxime and ceftraxon. They are also inhibited by clavulanate, and alone or in combination with β-lactams called β-lactam/β-lactamase inhibitors (Vinodhini *et al.,* 2014). Among the 9 different antibiotics used against all the isolates of *Enterobacteriaceae*, amikacin (73%) was found to be the less effective antibiotic which was in accordance with the similar study conducted by Ghimire (2016). Similarly in a study antibiotic sensitivity pattern of the isolates revealed that, 73% were sensitive to amikacin, whereas high resistance was seen for ampicillin (98.5%), ceftriaxone (73.55%), cefotaxime (72%), and ciprofloxacin (58%) (Metri *et al.* 2011). Of the total isolates of *Enterobacteriaceae*, 171 (85.5%) were found to be multidrug resistant. Similar study conducted in Sahid Gangalal Memorial Hospital by Ghimire (2016) showed 11.8% growth and 82.6% of them were MDR.

High drug resistance in *Enterobacteriaceae* is attributed to mutations in chromosomal genes ability to share genetic material and mobile resistant genes. The mobile genetic elements are responsible for capturing resistant genes from the chromosomes of a variety of bacterial species and then DNA molecules horizontally and vertically (Patridge 2015). Higher level of drug resistance seen in *Enterobacteriacae* is mediated by the production of different kind of beta-lactamases primarily ESBL, the fact that the carriage of resistance trait for quinolones and aminoglycoside in the plasmid along with the gene for beta-lactamases have had a great impact on the drug resistance character shown by this pathogenic bacteria (Upadhyaya, 2015). The distribution of specimens of *P. aeruginosa* may vary with each hospital as each hospital facility has a different environment associated with it. A study by Chander *et al.*, (2013). Increasing resistance to different anti-pseudomonal drugs particularly among hospital strains has been reported world-wide. In this study, the isolates showed highest resistance to Gentamicin (84%). the precious weapon against *P. aeruginosa* infections (even against MDR

isolates) which is an alarming sign. A recent study by mohanasaundaram (2011) with 193 *P. aeruginosa* showed 79% resistance to Gentamicin followed by Ceftriaxone (75%), Ciprofloxacin (73%), Ceftriaxon (63%) and Amikacin (41.5%) which correlates with our study. Our study, the rate of MRSA isolation was found to be 85.1% from clinical specimens. This result is higher than the many other studies conducted by Kumari *et al.* (26.14%), Shakya *et al.* (12.5%) and Tiwari *et al*. (69.1%) (Tiwari *et al.*, 2006; Kumari *et al.*, 2008; Shakya *et al.*, 2010). Amoxiclar was found resistant to all of MRSA isolates i.e. 100%. This result is higher than that of Shrestha *et al.* who reported 91.94% (Shrestha *et al.*, 2009). In present study, clinical MRSA isolates showed rate of resistance to antibiotics followed by Cloxacillin (94.7%), Cefotaxime (84.2%),All isolates were found to be multi drug resistant (MDR) in this study. The rate of MDR-MRSA (100%) is higher than that of the result reported in the studies conducted by Bhomi *et al.* i.e. 40.1% and Pandey *et al.* i.e. 75.86%. Though this study is in accordance with the previous studies from Nepal and other countries showing high percentage of MDR among MRSA; >65% by Kumari *et al.*, 93% by Rahimi *et al.* and 63% by Salah *et al.* (Salah *et al.*, 2012; Rahimi *et al.*, 2013).

REFERENCES

1. Angel, D. E.; Lloyd, P.; Carville, K. and Santamaria, N. The clinical efficacy of two semi-quantitative wound swabbing techniques in identifying the causative organism(s). Int Wound J., 2011; 8: 176–185.
2. Benbow, M. Wound swabs and chronic wounds. Practice Nurse, 2010; 39(9): 27–30.
3. Bowler, P. G.; Duerden, I. and Armstrong, D. G. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev., 2001; 14(2): 244–269
4. Brooks,G.F.; Butel, J.S.; Caroll, K.C. and Morse, S.A. Enteric Gram-negative rods (Enterobacteriaceae). In: Medical Microbiology. 24th Ed. 2007. Mc Graw Hill, 249-61.
5. Chakraborty D, Basu S and Das S. Study on some Gram negative multidrug resistant bacteria and their molecular characterization. Asian J Pharm Clin Res., 2011; 4: 108-112.
6. Chander A and Raza MS Antimicrobial susceptibility patterns of P. aeruginosa clinical isolates at a tertiary care hospital in kathmandu, Nepal.Asian J Pharm Clin Res., 2013; 6(3): 235-238.
7. Church D, Elsayed S, Reid O, Winston B and Lindsay R. Burn wound Infections. Clinical Microbiology Reviews, 2006; 19: 403-34.
8. Forbes, B. A.; Sahm, D. F. and Weissfeld, A. S. Bailey & Scott's Dignostic Microbiology 12th Edition: Mosby Elsevier, St. Louis. USA, 2007.

9. Garrity, G. Bergey's Manual of Systematic Bacteriology. (2nd Ed.). 2. Williams and Wilkins, Baltimore. London, 2005.
10. Ghimire A. Extended spectrum beta-lactamase (ESBL) producing multidrug resistant Gram negative bacteria from various clinical specimens of patients visiting in a tertiary hospital. M.Sc. Dissertation Submitted to Central Department of Microbiology. Tribhuvan University, 2016; 24-43.
11. Holt, J.; Krieg, N.; Sneath, P. and Williams, S. (1994). Bergey's Manual of determinative Bacteriology (9th Ed.). Williams and Wilkins. Maryland. USA.
12. Kumari N, Mohaprata TM and Singh I Prevalence of methicillin-resistance Staphylococcus aureus (MRSA) in a tertiary-care hospital in eastern Nepal. J Nepal Med Assoc, 2008; 47(170): 53-6.
13. Leboffe, M. J. and Pierce, B. E. A photographic Atlas for The Microbiology Laboratory. (4th Ed.), 2011; 65-95. Morton Publishing. Colorado.
14. Mayhall, C. G. The epidemiology of burn wound infections: then and now. Clin Infect Dis., 2003; 37: 543–550.
15. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S and Monnet DL Multi drug resistant, extensively drug resistant and pan drug resistant bacteria: an international expert proposal for interim standard defi nitions for acquired resistance. Clin Microbiol Infect, 2012; 18: 268-281.
16. Metri BC, Jyothi P and Peerapur BV The prevalence of ESBL among Enterobacteriaceae in a tertiary care hospital of North Karnataka, India. J Clin Diagn Res., 2011; 5: 470-475.
17. Mohanasoundaram KM The Antimicrobial resistance pattern in the clinical isolates of P. aeruginosa in a tertiary care hospital; 2008–2010 (A 3 Year Study). J Clin Diag Res., 2011; 5(3): 491-494**.**
18. Murray P. R., Baron E. J., Jorgensen J. H., Pfaller M. A., Yolken R. H., (Eds.), 8th Ed., 2003, Manual of Clinical Microbiology, ASM, Washington, D.C.
19. Obritsch MD, Fish DN, MacLaren R, Jung R. Pharmacotherapy, Oct, 2005; 25(10): 1353 64.
20. Sousa, A. M.; Machado, I.; Nicolau, A. and Pereira, M. O. Improvements on colony morphology identification towards bacterial profiling. Journal of Microbiological Methods, 2013; 95: 327–335.
21. Parajuli NP, Acharya SP, Mishra SK, Parajuli K, Rijal BP and Pokharel BM High burden of antimicrobial resistance among Gram negative bacteria causing healthcare associated infections in a critical care unit of Nepal. Antimicrob Resist Infect Control, 2017; 6: 67.

22. Patridge SR Resistance mechanisms in Enterobacteriaceae pathology. J RCPA, 2015; 47: 276-284.
23. Rahimi F, Bourazi M, Katouli M and Pourshafi e MR Antibiotic resistance pattern of methicillin resistant and methicillin sensitive Staphylococcus aureus isolates in Tehran, Iran. Jhundishapur J Microbiol, 2013; 6(2): 144-149.
24. Salah IK, Nagla AA and Ahmed IF Prevalence and antimicrobial susceptibility pattern of methicillin resistance Staphylococcus in Sudanese surgical ward. Pharmacology and Pharmacy, 2012; 3(1): 103-108.
25. Shakya B, Shrestha S and Mitra T. Nasal carriage rate of methicillin-resistant Staphylococcus aureus among at National Medical College Teaching Hospital, Birgunj, Nepal. Nepal Med Coll J., 2010; 12(1): 26-29.
26. Shrestha B, Pokhrel BM and Mohapatra TM Antimicrobial susceptibility pattern of nosocomial isolates of Staphylococcus aureus in a tertiary care hospital, Nepal. J Nepal Med Assoc, 2009; 48(147): 234-8.
27. Todar K., Todars Online Textbook of Bacteriology, University of Wisconsin - Madison, Department of Bacteriology.
28. Tiwari HK and Sen MR Emergence of Vancomycin resistant Staphylococcus aureus (VRSA) from tertiary care hospital from northen part of India. BMC infect Dis., 2006; 6: 156-160.
29. Upadhyaya U. Detection of beta-lactamase producing Gram negative bacteria in different clinical specimens of patients visiting tertiary level heart centre. M.Sc Dissertation Submitted to Central Department of Microbiology, Tribhuvan University, 2015; 28-36.
30. WHO-World Health Organization (2016) World Malaria Report 2013 Available from: who.int/malaria/ publications/world_ma laria_report_2016/en/.
31. Young, L. Identifying infection in chronic wounds. Wound Practice and Research: Journal of the Australian Wound Management Association. 2012; 20(1): 38–44.