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ORIGINAL ARTICLE

Biochemical Characterization and Antimicrobial Resistance of Bacterial Isolates from Post-Surgical Wounds in Jinnah Hospital Lahore, Pakistan

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Abstract

Objective: The current study was aimed to determine the antimicrobial sensitivity pattern of clinical isolates from post-surgical wounds.

Material and Methods: A retrospective study was carried out in Jinnah Hospital Lahore, Pakistan. Total of 150 post-surgical hospitalized subjects were enrolled. Specimen were collected from surgical site (wound). Cultured were done on Blood and MacConkey agar. Bacterial isolates were identified using morphological and biochemical characteristics. Antimicrobial susceptibility were assessed according to CLSI guidelines.

Result: Among 150 specimen 78 (25%) were wound swabs and 113 (75%) were pus specimen. Among cultured specimens, 115 (76.7%) were clinically significant. S.aureus, Pseudomonas, Acinetobacter and E. coli were isolated from these specimens. Staph aureus show (83.3%) resistant against Erythromycin, (50%) to penicillin, (50%) against Ciprofloxacin,(17%) resistant for Gentamicin, (9%) resistant for Methicillin. Acinetobacter species show resistant against Amikacin (33.3%), Ampicillin (44.4%), and Ciprofloxacin (77.8%) Erythromycin (33.3%), Tazobactum (42.9%), Imipenum (66.7%), Meropenum (55.6%), Augmentin (72.6%), Lenzolid (77.8%) resistant show. E coli were found resistant to Amikacin, Ampicilin, and Ciprofloxacin.

Conclusion: Bacterial infection is a significant problem in post-surgical patients admitted in hospital. Most prevalent microorganism are Staph-aureus, Pseudomonas, E.coli, and Acinetobacter. Multidrug resistance pose a life threatening risk to post-surgical patients.

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Introduction

Post-operative wound infections are main global problem in the field of surgery leading to many complications, increased morbidity and mortality.[1] Advances in control of infections have not completely eradicated the problem because of development of resistance. Infections acquired during hospital stay are also a significant public health problem across the world. Hospital acquired infections (HAI) contributed in high rates of mortality and morbidity.[2] Wound infections results in inflamed area with pus and contribute in fever and sickness. Wound infections can be resulted due to the normal flora of the microorganisms present on the skin or surface of the body or sometimes opportunistic organisms can cause infections. It has been observed that post-surgical wound infections are more problematic in patients with chronic diabetes, immunodeficient, obese or using any corticosteroids therapy.[3] By definition any infection that develops after 48hrs stay in hospital or after discharge from hospital is known as HAIs. These infections were not supposed to be present at the time of admission in hospital. Risk for wound infections is 5 to 10 times higher in intensive care patients admitted in General wards.[4] People with long stay in hospital have more chances of getting wound

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infection and at risk of life threatening infectious diseases.[5]

The rate of surgical site infection (SSIs) is reported in different studies which are estimated that the patients with surgical site infection (SSIs) have up to elevens times bigger risk of death as compared to the patients with infection other than SSI. Isolated Bacteria's from SSIs has not changed for the last decade because the most frequently isolated pathogens are still the same i.e. *Staphylococcus aurous, Coagulase negative Staphylococci, Enterococcus* specie and *Escherichia coli.*[6] It is stated that the specific therapeutic criteria for patients with post-surgical infections is dependent upon antimicrobial susceptibility tests generated by clinical laboratories in Hospital setup.[7] As the pattern of antimicrobial susceptibility is keep changing thus, the current study was aimed to determine the exact status of clinical isolates from post-surgical wounds and their antimicrobial sensitivity pattern in Jinnah Hospital Lahore, Pakistan.

Materials and Methods

A retrospective study was designed in Jinnah Hospital Lahore, Pakistan from January-2016 to june-2016. A total of 150 post-surgical hospitalized subjects were enrolled in the present study.

According to recommended guidelines and under aseptic conditions direct swabs and pus specimen from surgical site (wound) were taken from the patients. Pus specimen was collected by physician under aseptic condition. The end of a commercial sterile swab was moved over a minimum 1-centimeter area of stuff Swab was wet by normal saline and sufficient pressure was applied on the tip of the swab So that enough cellular material could be collected for culture after the removal of dressing and topical antimicrobial agent after cleaning of wound. Sample was collected after a week of admission in the hospital.

After proper labeling all specimens were transported immediately to Laboratory for Processing with no delay to avoid any contamination and before the swabs becomes dry.

Immediate Specimens were inoculated on Blood and MacConkey agars according to Clinical and Laboratory Standard Institution (CLSI) guidelines and incubated at 35 to 37°C for 18-24hrs. After 24hrs of incubation Colony morphology was recorded (color, size, margins, pigmentation and texture Concavity, convexity, Mucoid, Dry, and Swarming). Specimens containing mixed growth were subculture under aseptic conditions on Manitol salt agar Cysteine Lactose Electrolyte Deficient agar (CLED) and Chocolate ager. Incubation was done at 37°C for 18-24hrs in order to get isolated colonies.[8]

Bio Chemical Characterization

Gram Staining and Various biochemical tests that is Catalase test, DNase, Oxidase, Citrate test, Urease test, Motility, Indole, Triple Sugar Iron, MR and VP tests were performed on isolated bacterial colonies from culture for confirmations of sub-strains of bacteria.

Anti-Microbial Susceptibility Pattern

Mueller Hinton Agar was used for the evaluation of Anti-microbial susceptibility testing using Kirby-Bauer disc diffusion techniques. Bacterial isolates was prepared using 0.5 mc Farland standards and inoculated on Muller Hinton agar plate.[9] Plates were incubated for 24 hours at 37°C. After incubation diameter of the zone of inhibition were recorded in milli meter (mm). List of antibiotics used in the present study for susceptibility testing are shown in Table I.

Table I: Antibiotics used in this study according to CLSI guidelines

Antibiotics	Drug class	Dose (ug)
Amikacin	Aminoglycosides	30 (ug)
Ampicilin	Pencillin	10 (ug)
Augmenten	Pencillin	20 (ug)
Ciprofloxacin	Quinolones	05 (ug)
Erythromycin	Macrolids	15 (ug)
Vancomycin	Glycopeptides	30 (ug)
Tazobactum	Cepahalosporins	100/10 (ug)
Imipenum	Carbapenam	10 (ug)
Meropenum	Carbapenam	10(ug)
Cefuroxime	Cephalosporin	30 (ug)
Cefotazidime	Cephalosporin	30(ug)
Lanzolid	Lennzolid	30(ug)
Gentamycin	AminoGlycocides	10(ug)
Moxifloxacin	Flouroquinolone	05(ug)
Polymyxin B		300(ug)

Results

Total 150 patients were under the major operation admitted in surgical wards of Jinnah Hospital Lahore were enrolled in the present retrospective study, mostly patients were male 66.7% while only 33.3% were females. Mostly patients were in the age group 20-40 which is 91 (60.7%) as shown in Table II. Etiology of specimen include in this study revealed wound swabs 78 (25%) and pus specimen 113 (75%).

Table II: Demographic characteristic of patients

Demographic characteristics		**N**	**%**
Age	Less than 20 years	29	19.3
	20 – 40 years	91	60.7
	40 – 60 years	25	16.7
	More Than 60 years	5	3.3

Out of all the specimens cultured, 115 (76.7%) were having clinically significant growth while 35 (23.3%) were showing no growth on Cultural Media declared as Negative.

Bio Chemical Tests

On the basis of biochemical reactions, it was confirmed that 47 (41.59%) out of 115 cultured positive specimens were gram positive cocci strains while 68 (60.17%) were Gram Negative Bacilli, out of total Gram-positive cocci 39 were catalase positive (Staphylococcus specie) while only 7 were catalase negative (Streptococcus specie). Among the Gram-negative bacilli 39 were oxidase positive (*Pseudomonas species)* while 29 were oxidase negative. Among the oxidase negative gram negative bacilli, 22 were *E Coli* confirmed by TSI reaction and 07 were *Acinitobactor* species. Results showed that *Pseudomonas aeruginosa* was predominant microorganism among the others isolated microorganism from the post-surgical patients are given in Figure1.

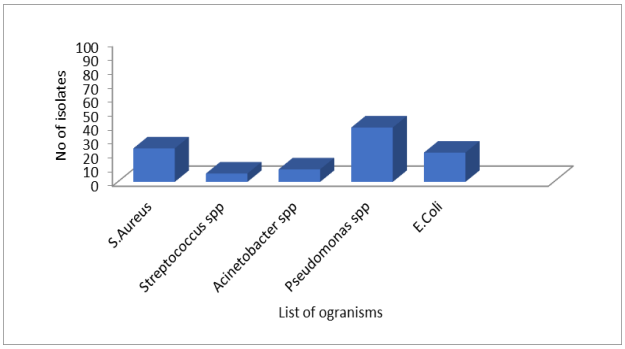

Figure 1. Frequency of isolated organisms

Antibiotic Resistant Pattern

Results of antimicrobial sensitivity pattern was obtained against *Staph aureus* which show that (83.3%) resistant against Erythromycin, (50%) against pencilin, (50%) against Ciprofloxacin, (17%) resistant for Gentamicin, (9%) resistant for Methicilin, so 9% Methicilin resistant *Staph aureus* were isolated from those patients as presented in Figure 2.

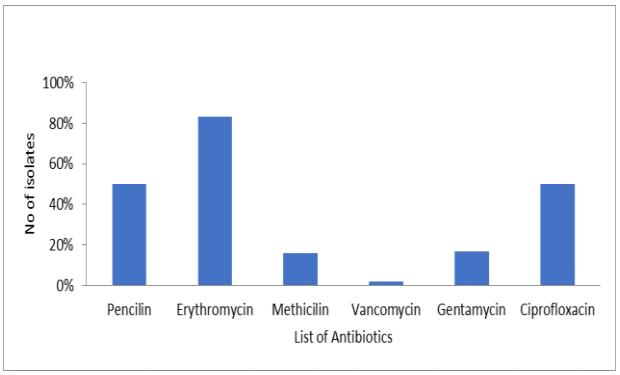

Figure 2. Antimicrobial resistant pattern of Staph aureus

E coli Show (19.0%) resistant against Amikacin, (14.0%) against Ampicilin, (57.1%) against Ciproflotacin, (47.6%) Tazobactam, (14.3%) Ciprofloxacin, (57.1%) Imipenum (47.2%), and Meropenun (47.2%) resistant show.

Acinetobacter species show resistant against Amikacin (33.3%), Ampicillin (44.4%), and Ciprofloxacin (77.8%) Erythromycin (33.3%), Tazobactum (42.9%), Imipenum (66.7%), Meropenum (55.6%), Augmentin (72.6%), Lenzolid (77.8%) resistant show.

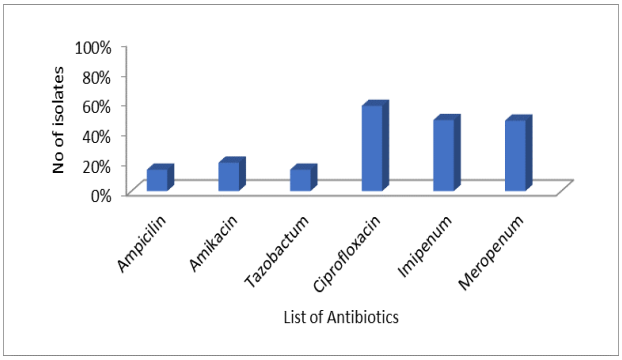

Figure 3. Antimicrobial resistant pattern in E coli.

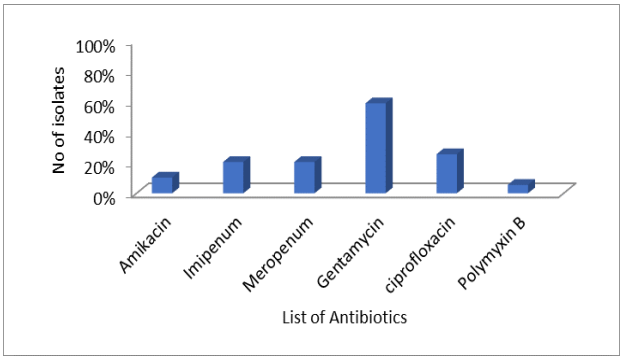

Figure 4. Antimicrobial resistant pattern in Pseudomonas.

Out of 39 isolated Pseudomonas species (10.3%) resistant against Amikacin, (20.5%) Imipenum, (20.5%) Moropenum, (59%) Gentamycin, (5.5%) resistant show to polymycin B.

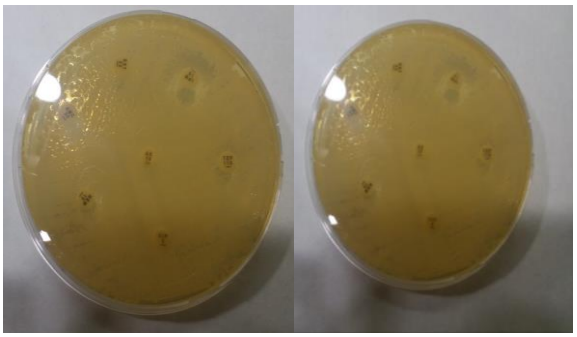

Figure 5. Antibiotic Resistance pattern of Acinetobacter

Discussion

This study was planned to find the frequency of nosocomial infection in post-surgical patients whose are admitted in surgical wards. One significant patron microorganism *Pseudomonas aeruginosa,* and second *staphylococcus aureus* was mostly isolated which was correlated with others investigations.[10]

The most widely recognized sorts of nosocomial contaminations that could happen in a healing center set up are surgical injury and other delicate tissue diseases,UTI, respiratory tract infection and circulatory system diseases.[11]

In nosocomial disease observation, there is no any significant change in the spread of pathogens listed from SSI for the current decade for *Staph. Aureus*, other Coagulase negative *staphylococci* and *enterococcus spp.* Moreover, *E-coli* are the most isolated pathogens from pathogenic specimens. The members of *Enterobacteraceae, Pseudomonas aureginosa, and streptococcus pyogenes* also add a significant burden to the nosocomial disease.[12]

In the present investigation, 150 specimens were analyzed, out of which 76.7% was cultured positive and 23.3% cultured was negative. Based on biochemical reactions and Gram staining It was further affirmed that out of 45 gram positive cocci strains, 38 were catalase positive while only 6 were catalase negative these catalase positive strain were furfures tried by coagulase test and DNase for affirmation of the Staph aureus species, and out of 68-gram negative strain 39 oxidase positive and 29 oxidase test negative. Based on various biochemical test it was affirmed that prevalent microorganism were Pseudomonas and second most generally creature was *Staph aureus* than other *E Coli*, *Acinitobactor* species.

Our study was supported by another examination in which 676 people were analyzed. 1060 bacterial strains were isolated from 676 people. A high prevalence of vigorous microbes was watched. Among the basic pathogens the most dominating microorganism is Staphylococcus aureus and second most regular pathogen is *Pseudomonas aeruginosa* than other isolated bacteria was, *E coli, Staphylococcus epidermis's,* Acinetobacter *spp and Enterococcus*. [13]

As *S. aureus* is a natural inhabitant of nasal cavity, the observed power (26.7%) of *S. aureus* may be due to contamination seen in as narrated by Isbori et al and Angu and Olila. The other sources contributing to its increased contamination may be the pollution from environment, surgical Instruments or contaminated hands and wearing hospital staff. In addition, in our investigation, *E. coli* (20%) was the second highest prevalent bacteria from SSI. The current study has also revealed that the vast majority of *S. aureus* were impervious to about every one of the medications tried with protection rate that reaches from 54.5% to 100. %. [14]

In our investigation Out of 39 secluded *Pseudomonas species* 59% Gentamycin 25.6% ciprofloxacin., 20.5% Imipenum, 20.5% Moropenum, 10.3% resistant Amikacin, 5.5% resistant show to polymycin B. *Pseudomonas aeruginosa*, in another examination, demonstrated high protection rates in fluoroquinolones (ciprofloxacin), ureidopenicillin (piperacillin) trailed by gentamicin, while least protection was seen against Amikacin and no seclude was observed to be Meropenem resistant. [15]

In this investigation *Staph aureus* indicated safe 83.3% safe for Erythromycin, half safe for Penicillin, half for Ciprofloxacin, 16.7% safe for Gentamycin, 9% safe for Methicillin were secluded. Another examination was done in India has likewise shown that a large portion of S. aureus were impervious to about every one of the medications tried with protection rate that reaches from 54.5% to 100%. This finding concurs with past investigations done locally by Messele G, Gedebou, Tesfahunegn and Mulu, where normal protection of 70.6%, 75%, 75% and 52% were acquired for the usually utilized anti-toxins, separately. [16]

General protection rate of Gram positive microbes to the regularly utilized anti-toxins in the present investigation ranges from 55– 75%; even up to 100% to Ampicillin. Iin India where 70% of thedetaches were impervious to Amoxicillin, Tetracycline, Chloramphenicol and Norfloxacin. In as opposed to the consequences of past investigations, *S. aureus* demonstrated slightest protection from Erythromycin (9.1%) and moderately high protection from Gentamycin (54.5%). [17]

In another investigation it was watched that at (FELEGE HIWOT REFERRAL HOSPITAL, BAHIRDAR, ETHIOPIA) Gram negative bacterial separates were fluidly safe for the medications tried. As showed in Figure 4, 90% of *E. coli* demonstrated resistance for Amoxacillin however not for Chloramphenicol. *P. aeruginosa* indicated 100% resistance for Ceftriaxone, Amoxicillin, Ampicillin and Nitrofurantoin. [18]

Antibiotic medication and Norfloxacin were drugs which moderately demonstrated low protection rate (20%) each for *P. aeruginosa. So also, P. mirabilis*, Gentamycin, Nitrofurantoin and Doxycycline, yet it was 100% touchy for ciprofloxacin and Kanamycin also most sensitive for *Pseudomonas aeruginosa.* In addition, *K. pneumonia,* which accounted 4.8% of the aggregate separate, indicated 100 % protection against Amoxicillin, Trimethoprim-sulphamethazole, Ciprofloxacin and Gentamycin however not to Tetracycline. Then again, Doxycycline was moderately the best medication for Proteus mirabilis, with no protection from this medication. [19]

In another study was done in UK results show Fifty seven (43.8%) of the segregated microscopic organisms were third-age Cephalosporin-safe. Protection from quinolones was seen in sixty-four (49.2%) microorganisms. Amoxicillin-clavulanate resistance was exhibited in 57 (43.8%) cases as shown in Figure 4. The 50/57 (88%) third-age cephalosporin-safe microorganisms indicated cross-protection from amoxicillin/clavulanate and the 42/57 (73.7%) were likewise impervious to quinolones Meropenem, the most incessant exactly endorsed anti-infection for HCA/nosocomial diseases, demonstrated a medication protection rate of 30.7%. Meropenem was ineffectual on both XDR microscopic organisms and E. *faecium.* Ten out of 13 (77%) XDR were vulnerable to colistin while all GPC including *E. faecium* and the 86% of GNB to tigecycline. Just 54% of the XDR were defenseless to tigecycline. Everything except one XDR microbes were conceivable blend of colistin and *tigecycline*. [20]

Conclusion

It is concluded that infection is a significant problemin post-surgical patientsadmitted in hospital.Most prevalent microorganism are *Staphaureus, Pseudomona, E.coli, and Acinetobacter*.Multidrug resistance is also big problem that contribute in life threatening situation in that patients.

References

1. Anguzu J, Olila D. Drug sensitivity patterns of bacterial isolates from septic post-operative wounds in a regional referral hospital in Uganda. African health sciences, (2007); 7(3).
2. Raza MS, Chander A, Ranabhat A. Antimicrobial susceptibility patterns of the bacterial isolates in post-operative wound infections in a tertiary care hospital, Kathmandu, Nepal. Open Journal of Medical Microbiology, (2013); 2013.
3. Carr W, Longo-Mbenza B, Apalata T, Moodley P, Sturm W. Association between symptomatic vulvovaginal candidiasis and HIV RNA levels in plasma and genital secretions among women on HAART. Southern African Journal of HIV Medicine, (2014); 15(2): 57-64.
4. Samuel S, Kayode O, Musa O, Nwigwe G, Aboderin A, et al. Nosocomial infections and the challenges of control in developing countries. African journal of clinical and experimental microbiology, (2010); 11(2).
5. Endalafer N, Gebre-Selassie S, Kotiso B. Nosocomial bacterial infections in a tertiary hospital in Ethiopia. Journal of Infection Prevention, (2011); 12(1): 38-43.
6. Giacometti A, Cirioni O, Schimizzi A, Del Prete M, Barchiesi F, et al. Epidemiology and microbiology of surgical wound infections. Journal of clinical microbiology, (2000); 38(2): 918-922.
7. Mengesha RE, Kasa BG-S, Saravanan M, Berhe DF, Wasihun AG. Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. BMC research notes, (2014); 7(1): 575.
8. Deepa N (2013) A Study on Microbiological Profile of Blood Stream Infections in patients admitted in Intensive Care Unit in a Tertiary Care Hospital: Madras Medical College, Chennai.
9. Donay J-L, Fernandes P, Lagrange P, Herrmann J-L. Evaluation of the inoculation procedure using a 0.25 McFarland standard for the BD Phoenix automated microbiology system. Journal of clinical microbiology, (2007); 45(12): 4088-4089.
10. Mulu W, Kibru G, Beyene G, Damtie M. Postoperative nosocomial infections and antimicrobial resistance pattern of bacteria isolates among patients admitted at Felege Hiwot Referral Hospital, Bahirdar, Ethiopia. Ethiopian journal of health sciences, (2012); 22(1): 7-18.
11. Kamat U, Ferreira A, Savio R, Motghare D. Antimicrobial resistance among nosocomial isolates in a teaching hospital in Goa. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine, (2008); 33(2): 89.
12. Ducel G, Fabry J, Nicolle L. Prevention of hospital acquired infections: a practical guide. Prevention of hospital acquired infections: a practical guide, (2002); (Ed. 2).
13. Kamat US, Ferreira AM, Savio R, Motghare DD. Antimicrobial resistance among nosocomial isolates in a teaching hospital in Goa. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2008 Apr;33(2):89.
14. Mengesha RE, Kasa BG, Saravanan M, Berhe DF, Wasihun AG. Aerobic bacteria in post surgical wound infections and pattern of their antimicrobial susceptibility in Ayder Teaching and Referral Hospital, Mekelle, Ethiopia. BMC research notes. 2014 Dec;7(1):1-6.
15. Gelaw A, Gebre-Selassie S, Tiruneh M, Mathios E, Yifru S. Isolation of bacterial pathogens from patients with postoperative surgical site infections and possible sources of infections at the University of Gondar Hospital, Northwest Ethiopia. Journal of Environmental and Occupational Health. 2014 May 21;3(2):103-8.
16. Onken A, Said AK, Jørstad M, Jenum PA, Blomberg B. Prevalence and antimicrobial resistance of microbes causing bloodstream infections in Unguja, Zanzibar. PloS one. 2015 Dec 23;10(12):e0145632.
17. Hu J, Ma XX, Tian Y, Pang L, Cui LZ, Shang H. Reduced vancomycin susceptibility found in methicillin-resistant and methicillin-sensitive Staphylococcus aureus clinical isolates in Northeast China. PloS one. 2013 Sep 12;8(9):e73300.
18. Fiore M, Maraolo AE, Gentile I, Borgia G, Leone S, Sansone P, Passavanti MB, Aurilio C, Pace MC. Nosocomial spontaneous bacterial peritonitis antibiotic treatment in the era of multi-drug resistance pathogens: A systematic review. World journal of gastroenterology. 2017 Jul 7;23(25):4654.
19. Jalan R, Fernandez J, Wiest R, Schnabl B, Moreau R, Angeli P, Stadlbauer V, Gustot T, Bernardi M, Canton R, Albillos A. Bacterial infections in cirrhosis: a position statement based on the EASL Special Conference 2013. Journal of hepatology. 2014 Jun 1;60(6):1310-24.
20. Latifpour M, Gholipour A, Damavandi MS. Prevalence of extended-spectrum beta-lactamase-producing Klebsiella pneumoniae isolates in nosocomial and community-acquired urinary tract infections. Jundishapur journal of microbiology. 2016 Mar;9(3).