

Nasal Carriage of Multi Drug Resistant *Staphylococcus aureus* among School Going Children of Kathmandu Valley

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ABSTRACT

Objectives: The present study was conducted to determine the multi drug resistant *Staphylococcus aureus* from nasal carriage among the school children of Kathmandu, Nepal.

Methods: This study was carried out from February to May 2018. A total of 100 nasal samples were collected from school going children of 7-15 years old. Nasal swabs collected were subjected to standard bacteriological techniques to identify *S. aureus* isolates. Antimicrobial susceptibility test was performed on Muller-Hinton Agar (MHA) by modified Kirby-Bauer disc diffusion method.

Results: Among 100 samples, 18% significant growth of *S. aureus* was found in which female had higher prevalence rate (18.03%) than male (17.95%). Prevalence was also high among age group 11-14 (19.44%), in private school (61.11%) compared to government school. Unhygienic practices like nose picking habit (77.78%), not covering nose while sneezing (55.56%), not using separate towel in family (88.89) were common. None of the isolates were MRSA and all showed resistance to amoxicillin and sensitive to chloramphenicol. Among 18 isolates 13 were MDR and they showed relatively high resistance to co-trimoxazole (44.44%) followed by ciprofloxacin (38.89%) and erythromycin (27.78%).

Conclusion: This study showed a high prevalence of MDR *S. aureus* carriage in school children indicating the spread of MDR *S. aureus* in the community.

Keywords: *Staphylococcus aureus*, antibiotic resistance, multi-drug resistance, school children, Nepal

INTRODUCTION

Staphylococcus aureus is one of the most common causes of hospital acquired and community acquired infections worldwide which results in substantial morbidity and mortality. Infection with *S. aureus* ranges from cutaneous infection, such as boils, impetigo to deep infections such as osteomyelitis, endocarditis, and bacteremia (Greenwood D, Slack R and Irving W 2012). *S. aureus* is normal flora of

human anterior nares, nasopharynx, perineal area, skin and can colonize various epithelial or mucosal surfaces. Spread of patient's endogenous strain to normally sterile site occurs by traumatic introduction (e.g., surgical wound or micro abrasions), from infected skin lesion of health care worker to patient, from person to person by fomites, air, or unwashed hands of health care workers, especially in nosocomial setting (Forbes 2007).

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S. aureus is a global health concern; however, it remains a normal commensal bacterium found in different body sites (Archer 1998; Paterson et al. 2014). Approximately one third of the human adult population carries this bacterium persistently yet asymptotically, while approximately 60% carry the bacterium intermittently. In contrast, some individuals remain un-colonized even after frequent exposure to *S. aureus* and they account for approximately 20% of the adult population (Melles et al. 2009; Chen et al. 2011; Brown et al. 2014). *S. aureus* can be transmitted between individuals easily via skin contact or contact with a contaminated environment (Brown et al. 2014).

The European Centre for Disease Prevention and Control (ECDC 2011) define MDR is defined as acquired resistance to at least one agent in three or more antimicrobial groups. It is an emerging problem in recent years. MDR bacteria are bacteria that have become resistant to certain commonly used antibiotics. There are diverse types of MDR bacteria that can be easily found throughout the environment including water and soil. They pose very little risk to healthy people, and some are carried as part of the normal bacteria in their bodies or in the skin.

The nasal carriers of *S. aureus* among school children in Kathmandu valley was reported 24.5% by (Joshi et al. 2003). Studies evaluating potential risk factors for CA-MRSA and nasal carriage status, however, are noticeably lacking. This study was conducted to determine the prevalence of *S. aureus* and MRSA in school children in Kathmandu valley and to evaluate the antibiotic susceptibility pattern of the isolates.

MATERIALS AND METHODS

Sample collection

A total 100 samples of nasal swab from anterior nares of student were collected from different school of children of age group <15 years of Kathmandu valley. The time duration for the work to complete was 4 months from February to May 2018. Written questionnaires were collected which contained information such as practices like nose picking, separate towel for family members, hand washing, closeness to or relationship with the health workers etc. For the collection of samples, a sterile cotton-tipped swab was moistened in normal saline and swirled

inside both anterior nares and was rotated in clockwise and anticlockwise direction (Louisiana Office of Public Health Infectious Diseases Epidemiology). All the collected nasal swabs were dipped in sterile normal saline and kept in an ice box and transported to the laboratory of the Padma Kanya Multiple Campus as soon as possible.

Identification of *S. aureus*

Primary inoculum was made in Mannitol Salt Agar (MSA). Golden yellow color colonies from MSA were sub-cultured on Nutrient Agar at 37°C for 24 hours (Photograph 1). Gram staining was performed in which gram positive i.e., purple organism was seen in cocci shape with cluster arrangement (Photograph 2). Different biochemical test like catalase, oxidase, O/F, DNase (Photograph 3), and coagulase were performed for the confirmation of *S. aureus*.

Antibiotic Susceptibility Test of *S. aureus*

Kirby Bauer disc diffusion method according to CLSI 2014 was employed to determine the susceptibility to antibiotic discs where four to five similar colonies were transferred to Nutrient Broth and the suspension was compared with 0.5% McFarland standards. Antibiotics like Amoxicillin (10 µg), Cefoxitin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (5 µg), Co-trimoxazole (25 µg) and Erythromycin (10 µg) were used (Photograph 4).

Data analysis

The results were recorded, and analysis was done using SPSS. Chi-square test was employed for evaluation with significance level of 0.05 for statistical analysis.

RESULTS

Distribution of *S. aureus* by gender

A total of 100 nasal swabs (61 females and 39 males) were examined and out of these in 18 (18%) samples significant growth was found. Female had higher incidence rate 18.03% (11/61) than male 17.95% (7/39) with no significant difference (P>0.05).

S. aureus isolation by different age groups

The prevalence of *S. aureus* was highest among age group below 10 i.e., 7-10 years (19.44%, 7/36) followed by age group above 10 i.e., 11-14 years (17.19%, 11/64). It was found that, there was no significant difference between prevalence of *S. aureus* and age group.

Occurrence of *S. aureus* according to type of school

Out of 100 samples collected 50 were from government school and 50 were from private school. 11(61.11%) isolates were from private and 7 (38.89%) were from government school. Growth was higher in private school than in government, though not significant statistically.

Association between occurrence of *S. aureus* and personal hygiene

41 students have nose picking habit. The prevalence of *S. aureus* was high among children with nose picking habit (77.78%, 14/18) than those children who doesn't have nose picking habit (22.22%, 4/18) ($p < 0.05$).

84 children had habit of covering nose while sneezing. The prevalence of *S. aureus* was high among children who do not cover nose (55.56%, 10/18) than who cover nose (44.44 %, 8/18). There was significant relation between habit of

covering nose while sneezing and significant growth. Only 34 children had separate towel for their family member. The prevalence of *S. aureus* was high among those who doesn't have separate towel for their family member (88.89%, 16/18) than those who have separate towel for their family (11.11%, 2/18). There was significant difference between use of separate towel in family and significant growth.

Antibiotic susceptibility pattern of *S. aureus* isolates

Table 7 shows antibiotic susceptibility of *S. aureus* towards different antibiotics. It was found that all the isolates of *S. aureus* were amoxicillin resistance whereas cefoxitin and chloramphenicol sensitive.

Distribution of Multidrug Resistant (MDR) *S. aureus*

Out of 18 isolates, 13 (72.22%, 13/18) were MDR *S. aureus* and 5 (27.78%, 5/18) were non MDR *S. aureus* (Figure 1).

Table-1: Distribution of *S. aureus* among male and female in study groups

Gender	Total (N)	Significant growth (N)	Percentage (%)	P-value
Male	39	7	17.95	
Female	61	11	18.03	P>0.05
Total	100	18	18	

Table2: *S. aureus* isolated from different age group among study groups

Age group	Total (N)	Significant growth (N)	Percentage (%)	P-value
Below 10	36	7	19.44	
Above 10	64	11	17.19	P>0.05
Total	100	18	18	

Table 3: Occurrence of *S. aureus* according to type of school

Type of school	Total (N)	Significant growth (N)	Percentage (%)	P-value
Government	50	7	38.89	
Private	50	11	61.11	P>0.05
Total	100	18	18	

Table-4: Significant association between occurrence of *S. aureus* and nose picking habit

Nose picking habit	Total (N)	Significant growth (N)	Percentage (%)	P-value
Yes	41	14	77.78	P<0.05
No	59	4	22.22	
Total	100	18	18	

Table-5: Distribution of *S. aureus* based on habit of covering nose while sneezing

Cover nose while sneezing	Total (N)	Significant growth (N)	Percentage (%)	P-value
Yes	84	8	55.56	P<0.05
No	16	10	44.44	
Total	100	18	18	

Table 6: Incidence of *S. aureus* in relation with using separate towel in family

Separate towel in family	Total (N)	Significant growth (N)	Percentage (%)	P-value
Yes	34	2	11.11	P<0.05
No	66	16	88.89	
Total	100	18	18	

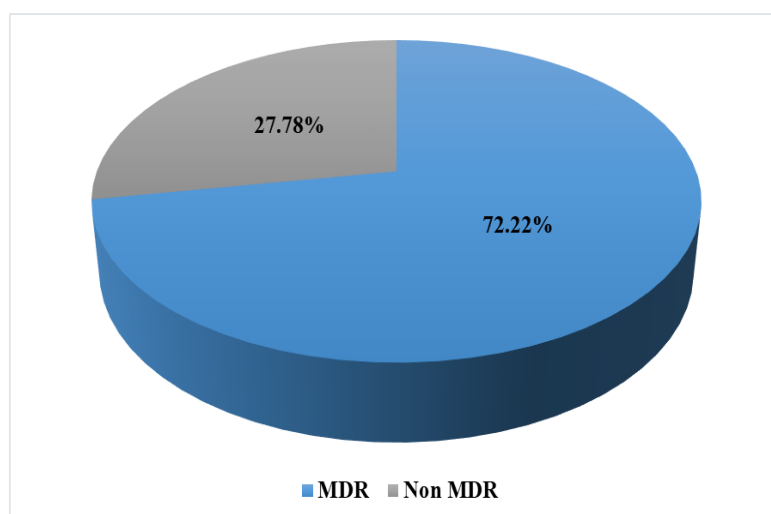


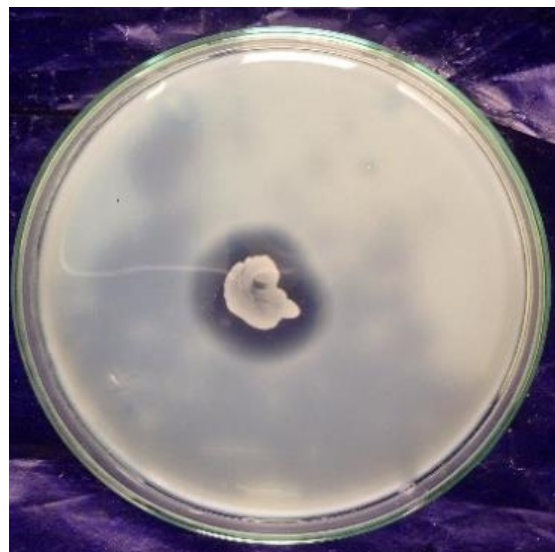
Figure 1: Distribution of Multidrug Resistant (MDR) *S. aureus* from study population

Table 7: Antibiotic susceptibility pattern of *S. aureus* isolates

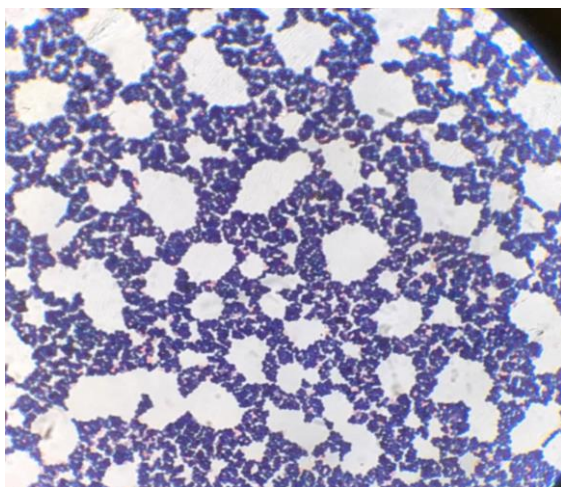
Antibiotics	Total <i>S. aureus</i> (N)	Sensitivity		Resistance	
		Number (N)	Percentage (%)	Number (N)	Percentage (%)
Amoxycillin (10mcg)	18	0	0%	18	100%
Chloramphenicol (30mcg)	18	18	100%	0	0%
Co-trimoxazole (25mcg)	18	8	44.44%	10	55.56%
Ciprofloxacin (5mcg)	18	7	38.89%	11	61.11%
Erythromycin (10mcg)	18	5	27.78%	13	72.22%



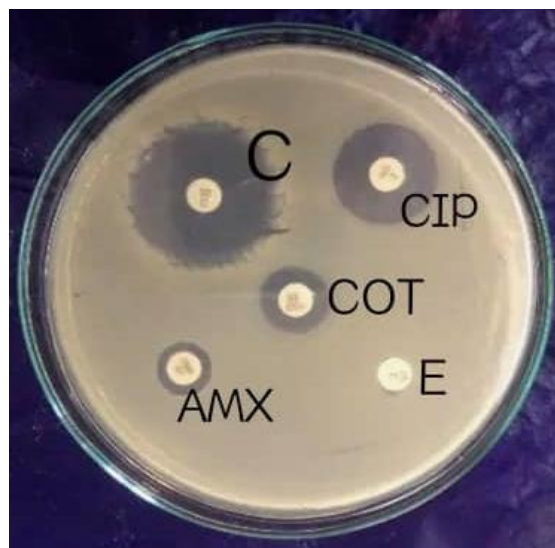
Photograph 1: Mannitol Salt Agar showing yellow colonies of *S. aureus*



Photograph 3: DNase test (positive)



Photograph 2 : Gram Staining of *S. aureus*



Photograph 4: Antibiotic susceptibility of *S. aureus*

DISCUSSION

A total of 100 samples were taken from anterior nares for this study which showed an overall prevalence 18% of *S. aureus* in the nostrils of school going children of both male and female which is less than the previous findings of Joshi et al. (2003) who reported a nasal colonization of 24.5% among school children in Kathmandu valley. However, in the study conducted in Kathmandu by Poudel et al. (2008) to determine the prevalence of *S. aureus* in school children, it was found that 11.8% had *S. aureus* isolated from nasal swabs. Further, the global rates have been reported to vary from 18 to 50% in different populations and the carriage of *S. aureus* in the nose appears to play a vital role in the epidemiology and pathogenesis of infection (Kluytmans et al. 1997). These differences might be due to different environmental, climatic, and geographical condition of the study place.

In this study, female had higher prevalence rate of *S. aureus* 18.03% (11/61) than male 17.95% (7/39). This means that sex is a risk factor for nasal colonization of *S. aureus* and the activity of the female groups predisposes them to *S. aureus* colonization or infection which is in contrast with the study of Rijal et al. (2008) in which there was no significant sex difference in colonization of *S. aureus*.

The prevalence of *S. aureus* was highest among age group 7-10 years 17.19% (11/64) followed by age group 11-14 years 19.44%, (7/36). It was found that, there was significant difference between prevalence of *S. aureus* and age group. This result was in agreement with the previous study of Kuehnert (2006) in which *S. aureus* colonization prevalence was highest in participants of 6-11 years old. Children may contribute differently than adults to the transmission of *S. aureus* through their behavior or vulnerability. Children (aged 0-17 years) might be colonized with *S. aureus* more commonly and for longer than adults Halablab et al. (2010). The prevalence of *S. aureus* in nasopharynx in small children is quite stable between 20% and 30% until it jumps to 40-50% and remains there from age 6 to 12 years of age after which it gradually decreases down to approximately 25% at age 18 Bogaert et al. (2004). The high mean isolates of *S. aureus* in this age group could be because of lower immunity power against infection and due to body contact, such as hugging and handshakes which young adults usually indulge in.

Body contact is one of the means through which *S. aureus* is transmitted in the community.

In case of nose picking habit of children, the prevalence of *S. aureus* was high among children having nose picking habit (77.78%, 14/18) than those children who doesn't have nose picking habit (22.22%, 4/18). There was significant difference between children having nose picking habit and children who doesn't have nose picking habit. The result was in agreement with the result of Wertheim et al. (2006) in which, nose pickers were significantly more likely than non-nose pickers to carry *S. aureus*. Their study came up with the conclusion that nose picking is associated with *S. aureus* nasal carriage and the role of nose picking in nasal carriage may well be causal in certain cases. Breaking the practice of picking nose could help *S. aureus* decolonization strategies. A typical transmission route of *S. aureus* is from the nose to the hand of a person (Wertheim et al. 2006) then to a surface (e.g. a door knob), and/or via the hand to the nose of a second person.

Out of 100 total samples, 84 children have habit of covering nose while sneezing. The prevalence of *S. aureus* was high among children who do not cover nose (55.56%, 10/18) than who cover nose (44.44 %, 8/18). The act of sneezing is a unique rifle and is a common manifestation of both colds and nasal allergies. Sneezing has also been interpreted as a favorable sign in numerous cultures (Rosner F 1999). A study by Werner et al. (2006) revealed that sneezing caused a 4.7-fold increase in the airborne dispersal of *S. aureus*, a 1.4-fold increase in coagulase-negative staphylococci (CoNS), and a 3.9-fold increase in other bacteria. Thus, covering nose while sneezing does not let in the dispersal of *S. aureus* through air and leading to low prevalence of the bacteria.

In this study, out of 100 samples collected 50 were from government school and 50 were from private school. Among 18 significant growths 11(61.11%) were from private school and 7 (38.89%) were from government school. Significant growth was seen high in private school than in government school. But statistically there was no relation between type of school and significant growth. According to the finding of this study the type of school cannot be considered as the risk factor for the occurrence of *S. aureus*.

The prevalence of *S. aureus* was high among those who doesn't have separate towel for their family member (88.89%, 16/18) than those who have separate towel for their family (11.11%, 2/18). There was significant difference between use of separate towel in family and significant growth. High contamination rates at frequently touched areas may play a significant part in indirect household transmission such as fomites include hand towels which plays role in transmission of infection (Scott et al. 2008). Modifiable behaviors, such as sharing personal items, may contribute to transmission.

All the isolated strains of *S. aureus* were evaluated with specific antibiotics by using Kirby Bauer Disc diffusion method. The isolated *S. aureus* were categorized into two groups. Methicillin Sensitive *S. aureus* (MSSA) and Methicillin Resistant *S. aureus* (MRSA). Non-Methicillin resistant strain of *S. aureus* are called Methicillin Sensitive *S. aureus* (MSSA). Antibiotic susceptibility pattern of *S. aureus* isolates showed that out of total 18 isolates from 100 samples it was found that all the isolates of *S. aureus* i.e., 100% were amoxicillin resistance whereas cefoxitin and chloramphenicol sensitive. 44.44% of isolates were sensitive as well as resistance to co-trimoxazole i.e., equal strains of both sensitive and resistance *S. aureus* was found towards co-trimoxazole. 38.89% of isolates showed sensitivity and 55.56% were resistance to ciprofloxacin. 27.78% of isolates were sensitive to erythromycin and 72.22% were resistance strains.

It has been observed that the most effective drug for *S. aureus* is chloramphenicol (100%), followed by co-trimoxazole (44.44%), ciprofloxacin (38.89%) and erythromycin (27.78%). All the isolates were sensitive to methicillin i.e., Methicillin Sensitive *S. aureus* (MSSA). MRSA strains were not isolated as all the isolates showed sensitivity to cefoxitin. All the MDR strains were subjected to cefoxitin antibiotic which if showed zone of inhibition less than or equal to 21mm would be confirmed as MRSA. As all the isolates showed zone of inhibition greater than 21mm it was confirmed that all MDR isolates were MSSA.

The susceptibility test results showed ampicillin to be the least effective agent with 100% bacterial resistance. This is basically because of beta-lactamases produced by *S. aureus*. Cleavage of the β -lactam ring by β -lactamases/penicillinases and alterations in the target PBPs

that reduce their affinity to the penicillin's are the worthiest cause of *S. aureus* resistance to penicillin group of antibiotics. *S. aureus* resistance (72.22%) to erythromycin, which is tetracycline group of antibiotics is greater than its sensitivity (27.78%) is due to active efflux of the antibiotic out of the cell. The uncontrolled availability of the agent in every drug vendor, which leads to its frequent use and misuse exert greater selection pressure for the resistant strains thereby makes this agent almost useless in the treatment of staphylococcal infections (Okeke 1999).

The indiscriminate and irrational use of antibiotics before full course is one of the major causes of emergence of antibiotic resistance in our country. Lack of antimicrobial surveillance within hospital premises, ineffective hospital decontamination procedure and unhygienic practice has led to spread of antibiotic resistant *S. aureus* strain within ward and community.

Multi- drug resistant (MDR) *S. aureus* were identified by their antibiotic sensitivity pattern. The *S. aureus* resistant to two or more than two classes of commonly prescribed antibiotics were considered as MDR. Multi- drug resistant (MDR) was defined as acquired non susceptibility to at least one agent in three or more antimicrobial categories (CLSI 2009). Out of 18 significant growths, 13 (72.22%, 13/18) were MDR *S. aureus* and 5 (27.78%, 5/18) were non MDR *S. aureus*. Prevalence of MDR *S. aureus* was high in our sample. 6 antibiotics namely Amoxycillin, Erythromycin, Chloramphenicol, Co-trimoxazole, Ciprofloxacin and Cefoxitin were used. Among all 18 isolates, all were resistant to amoxicillin, 13 were resistant to erythromycin, and 8 resistant strains to co-trimoxazole were found. Similarly, 10 resistant strains to ciprofloxacin were found. All the isolates were sensitive to chloramphenicol and cefoxitin.

In Nepal, purchasing antibiotics does not require a doctor's prescription. Anyone can directly purchase antibiotics from pharmacies. This lack of legislation for prudent use of antibiotics leads to indiscriminate and irrational use of antibiotics without prescription. Further, there is a knowledge gap regarding the cons of termination of antibiotics before full course which is one of the major causes of global emergence of MDR organisms. Additionally, there is a scant public awareness regarding the detrimental consequences of the increase in antimicrobial resistance

bacteria, which result in individuals using antibiotics as desired and contributing to the problem.

CONCLUSION

Prevalence of *S. aureus* was found to be 18% according to this study. Colonization of *S. aureus* was found to be related with certain type of health hygiene of children and their family. Therefore, it is crucial to educate parents and children about the importance of developing healthy habits. Further, out of 18 positive samples in this study 13 samples were MDR and 5 were non-MDR which concluded that MDR *S. aureus* has been growing. To reduce the emergence in antimicrobial resistance, proper awareness should be spread.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

REFERENCES

Adhikari and Sharma (1998). Antimicrobial Activities of some plant extracts. *J Nat Hist Mus* **11** (1-4): Nepal

Ahmad, I; Mehmood, Z; Mohammad. F (1998) Screening of some Indian Medicinal Plants for their Antimicrobial Properties. *J Ethnopharmacol* **62** (2): 183-193

Ahmad, V.U.; Khaly Vz-Zaman. S.M.; Ali. M.S.: Parveen. S.: Ahmad, W (1999) Antimicrobial ecdysone from *Asparagus dumosus*. *Fitoterapia* **67**(1): 88-91.

Ahmed El-sawi. S.; F. Abd El-Megled Hashem and Ali A.M. (1999) Flavonoids and Antimicrobial volatiles from *Adhatoda vasica* Nees. *Pharm Pharmacol Lett* **9**(2): 52-56.

Alasbahi, R.H. Safiyera, S. and Craker, L.E. (2001) Antimicrobial activity of some Yemeni Medicinal Plants. *J Herbs Spices Med Plants* **6**(3): 75-83.

Balows. A.; Hausler Jr, W.J.; Herrmann. K.L: Isenberg, H.D. and Jenschadomy. H (2011) *Manual of Clinical Microbiology* 5th edition, American Society for Microbiology. Washington DC, USA.

Barmet. H (2002) *The Natural Pharmacy, An Encyclopedia Illustrated Guide to Medicine from Nature*. Mirriampolunin and Christopher Robins. Great Britain.

Baron EJ. Peterson, LR and Finegold SM (2004). Bailey and Scott's *Diagnostic Microbiology* 9th edition Mosby-Year Book, Inc. USA.

Baur AB, Kirby WMM, Sherris KC and Truck M (2007). Antibiotic Susceptibility Testing by Standardized Single Disc Method. *Am J Clin Pathol* **44**: 493-496.

Bhattacharjee; SK. (1998) *Hand Book of Medicinal Plants*. 1st edition, Pointer Publishers. Jaipur India.

Brantner, AH. and Chakraborty, A (2008) In vitro antibacterial activity of alkaloids isolated from *Adhatoda vasica* NEES. *Pharm Pharmacol Lett* **8**(3): 137-139.

Caceres, A., Cano. O. Samayoa, B. and Aquilar, H. (1990) Screening of 84 plants, used in Guatemala for the treatment of Gastrointestinal Disorder, Against Enterobacteria. *J Ethnopharmacol* **30**(1): 55-73

Cai, L. and Wu. C.D: (2014) Compounds from *Syzygium aromaticum* possessing growth inhibitory activity against oral pathogens. *J Nat Prod* **59** (9): 897-990.

Carpinella. M.C; Herrero. G.G.; Alonso, R.A. and Palacios. S.M. (2009) Antifungal Activity of *Melia azedarach* Fruit Extract. *Fitoterapia*. **70**(3): 296-298.

Carpinella. MC; Herrero. GG. Alonso, R.A. and Palacios. S.M (2003) Antifungal Activity of *Melia azedarach* Fruit Extract. *Fitoterapia* **70**(3): 296-298.

Chakraborty. P. (1996) *A Text Book of Microbiology* 1st edition reprint. New Central Book Agency (P) Ltd. Calcutta, India.

Chandel. BS, Shah NM and Tripathi RM (2000) In-vitro Antibacterial activity of *Acacia arabica* bark. *Indian J Indig Med* **9**(1&2): 77-79.

Chattopadhyay D Sinha BK. & Vaid. LK. (2008) Antibacterial activity of *Syzygium* species. *Fitoterapia*. **69**(4): 365-367.

Chatwal. G.R. Anand. S. (2010) Instrumental Methods of Chemical Analysis. Edited by Arora. M. and Puri. S. Himalaya Publishing House, India.

Cheesbrough M (2013). *Medical Laboratory Manual for Tropical Countries*. Vol. II ELBS.

- Chopra. RN, Nayer, SL (2000) *Glossary of Indian Medicinal Plants*. PID, New Delhi.
- Chopra RN, Chopra IC., Handa KL and Kapur LD (1994) *Chopra's Indigenous Drugs of India* 2nd edition Academic Publishers.
- Chopra RN, Nayer SL and Chopra IC (1998) *Glossary of India Medicinal Plants*. CSIR New Delhi.
- Chopra RN, Nayer SL, Verma BS (2012) *Suppliment to Glossary of Indian Medicinal Plants*. Publications and Information Directorate.
- Choudhary MH (2005). Antibacterial activity of the root bark of *Holarrhena antidysenterica*. *Bangladesh J Microbiol* **15**(2): 67-70.
- Lee CK (2005) Screening and isolation of antibiotic resistance inhibitors from herb materials IV- resistance inhibitors for *Ameranthem graveolens* and *Acorus gramineus*, *Arch Pharmal Res* (Seoul) **21**(6): 734-737.
- Clark AM (2004). Natural Products as a Resource for New Drugs. *Pharm Res* (New York) **13**(8):1133-1141.
- Cleeland R and Squires E (2006). Evaluation of New Antimicrobials In Vitro and in Experimental Animal Infections. *Antibiotics in Laboratory Medicine*. 3rd edition by Victor-Lorian, Published by Williams and Wilkins, Baltimore.
- Clinical and Laboratory Standard Institute (CLSI 2018). Performance standards for antimicrobials susceptibility testing: 24th informational supplement (M100-S23). CLSI, Wayne PA, USA.
- Collee JE, Barrie DM., Andrew GF and Anthony S (2013) Mackie and McCartney *Practical Medical Microbiology* 14th Edition, Churchill Livingstone.
- Cruz AB, Moretto E, Filho VC, Niero R, Montanasi JL and Yunes RA (2013) Antibacterial activity of *Phyllanthus urinaria*. *Fitoterapia* **65**(5): 461-462.
- Deans, SG, Noble RC, Hiltunen R, Wuryani, W and Penzes LG (2005). Antimicrobial and antioxidant properties of *Syzygium aromaticum* (L) Merr and Perry: Impact upon bacteria, fungi and fatty acid levels in ageing mice. *Flavour and Fragr J* **10**(5):323-328.
- Devkota, KP, Acharya R., Baral MP and Adhikari. RP (2010). Antimicrobial Activities of Some Herbal Plants used in Traditional Medicine in Nepal. Proceedings of 3rd National Conference on Science and Technology March 8-11, Vol II: 1311-1317.
- Dingle J, Red WW and Solomons GL (2012). The enzymatic degradation of pectin and other polysaccharides II Application of the cup assay method to the estimation of enzyme. *J Sci Food Agric* **4**:149-153.
- Dixit. S.N. and Tripathi. S.C. (2015) Antifungal Antibiotics from Higher Plant 519-523 *Recent Advances in the Biology of Microorganisms*. Part II Editors: K.S. Bilgrami and K.M Vyas.
- Dijipa. C.D.; Michel. D. and Joelle. Q.L. (2000) Antimicrobial activity of bark extracts of *Syzygium jambos* (L) Alston (Myrtaceae), *J Ethnopharmacol* **71** (1-2): 307-313.
- Dulger. B.; Murat. C.; Moustafa. A. and Emin. U. (2015) Antimicrobial activity of *Artemisia absinthium* L. *Turk J Biol* **23**(3):377-384.
- Ebi. G.C. and Ofoefule. S.I. (2000) Antimicrobial activity of *Pterocarpus osnustems*. *Fitoterapia* **71**(4):433-435.
- Maharjan R, Thapa S, Acharya A (2019). Evaluation of Antimicrobial Activity and Synergistic Effect of Spices against Few Selected Pathogens. *TUJM* **6** (1):10-18.
- Sakha H, Hora R, Shrestha S, Acharya S, Dhakal D, Thapaliya S, Prajapati K (2018). Antimicrobial Activity of Ethanolic Extract of Medicinal Plants against Human Pathogenic Bacteria *TUJM* **5**(1): 1-6.