Journal of Medical Genetics and Genomics

Volume 3 Number 3 March, 2011
ISSN 2141-2278

Academic Journals

ABOUT JMGG

The **Journal of Medical Genetics and Genomics (JMGG)** is published monthly (one volume per year) by Academic Journals.

Journal of Medical Genetics and Genomics (JMGG) is an open access journal that provides rapid publication (monthly) of articles in all areas of the subject such as metagenics, evolutionary anthropology, fragile X syndrome, immunotherapy etc.
The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles published in JMGG are peer-reviewed.

Submission of Manuscript

Submit manuscripts as e-mail attachment to the Editorial Office at: jmgg@acadjournals.org. A manuscript number will be mailed to the corresponding author shortly after submission.

The Journal of Medical Genetics and Genomics will only accept manuscripts submitted as e-mail attachments.

Please read the **Instructions for Authors** before submitting your manuscript. The manuscript files should be given the last name of the first author.

Editors

Editorial Board

Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The **cover letter** should include the corresponding author's full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author's surname, as an attachment.

Article Types

Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process

All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review.

Decisions will be made as rapidly as possible, and the journal strives to return reviewers' comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the JMGG to publish manuscripts within weeks after submission.

Regular articles

All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors' full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length.. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited.

Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard **Abbreviations** should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.

Results should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors' experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The Discussion should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The Acknowledgments of people, grants, funds, etc should be brief.

Tables should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

Figure legends should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

References: In the text, a reference identified by means of an author‘s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author‘s name should be mentioned, followed by ’et al‘. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ’a‘ and ’b‘ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001)

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

Short Communications

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.

Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the Journal of Medical Genetics and Genomics is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Journal of Medical Genetics and Genomics

Table of Contents: Volume 3 Number 3 March 2011

ARTICLES

0.922

0.852

0.628

Journal of Medical Genetics and Genomics Vol. 3 (3) pp. 41 - 45, March 2011
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2011 Academic Journals

Full Length Research Paper

Detection of (*mecA*)gene in methicillin resistant *Staphylococcus aureus* (MRSA) at Prince A / Rhman Sidery Hospital, Al-Jouf, Saudi Arabia

Meshref Awad Al-Ruaily and Omer Mohamed Khalil*

Department of Clinical Microbiology, Al-Jouf University, Saudi Arabia.

Accepted 24 January, 2011

The study was conducted in A/ Sidery Hospital Al-Jouf province, Saudi Arabia during, the period September 2008 to May 2009. A total of 930 patients attended the Hospital for routine investigations were screened for urinary tract infection. A total of nine hundred and thirty urine specimens were received in the laboratory for culture, 640 specimens showed significant growth from which 100 *Staphylococcus aureus* species were recovered. Disk diffusion method and BD phoenix TM system antibiotic susceptibility were used, 15 methicillin resistant *S. aureus* isolates were identified, polymerase chain reaction (PCR) was used to amplify both the *S. aureus* specific sequence gene and *mec*A gene with the amplicon size of 107 and 532 bp. All the isolates (n=100) expressed *S. aureus* specific sequence gene in their PCR products. The results of the PCR revealed 13/15 isolates demonstrating both resistance to methicillin and expression of *mec*A gene, while the remaining two showed the resistance to methicillin by the disc diffusion method without the expression of *mec*A gene. All the isolates (n = 100) were sensitive to vancomycin.

Key words: *mecA*, MRSA, methicillin resistance polymerase chain reaction (PCR), Staphylococcus *aureus*, Al-Jouf, Saudi Arabia.

INTRODUCTION

Staphylococcus aureus is one of the most frequent bacterial pathogens in humans. It causes skin infections, osteoarthritis and respiratory tract infections in the community, as well as postoperative and catheter-related infections in hospitals (Didier et al., 2004) Methicillin-resistant *S. aureus* (MRSA) has become a major public health problem worldwide (Jarvis et al., 2007). The burden of MRSA continues to rise, with a growth rate of 14% of all *S. aureus* strains from clinically significant samples in New South Wales, Australia (Nimmo et al., 2006). The rising colonization rates lead to the increasing of infection rates in the community and in hospitals. The consequence to the health care system is longer hospital stays and greater costs, which approximately double the expenditure per patient (Kim et al., 2001*).* The patient risks include significantly higher mortality and morbidity rates with invasive MRSA infection (Lodise and McKinnon, 2005, Kearns et al., 1999) Within U.S. hospitals, nearly 60% of nosocomial *S. aureus* infections acquired in intensive care units are methicillin resistant (NNIS, 2004). Health care workers may carry MRSA on their hands or clothes following their contact either with to asymptomatic carriers or patients who have clinical infection. Health workers may then, unknowingly transmit the organism to other patients. The contaminated environmental surfaces also contribute to the MRSA transmission. Thus, symptomatic patients constitute a small portion of the actual reservoir of MRSA within hospitals resulting in an iceberg phenomenon (Harbarth et al., 2000). The world wide emergence of community acquired methicillin resistant *S. aureus* (CA-MRSA) can have severe public health implications (Calfee et al., 2003). The differentiation between community-acquired MRSA and hospital acquired MRSA (HA-MRSA) is becoming difficult to understand, since CA-MRSA could spread into hospitals (Wannet et al., 2004). The risk of

*Corresponding author. E-mail: omarkalil2008@gmail.com.

the acquiring MRSA in the hospitals increased by severity of illness (Ibelings and Bruining, 1998), length of stay, (Law and Gill., 1998), use of intravascular devices (Pujol et al., 1994) and the intensity of exposure to infected patients (Merrer et al., 2000). Infection control measures include screening, (Girou et al., 1998) and (Lucet et al., 2003) segregation of positive patients, (Arnoldet et al., 2002), eradication of carriage (Hill et al., 1988) and good standards of general hygiene (Rampling et al., 2001).

MATERIALS AND METHODS

The study was conducted in A/ Sidery Hospital Al-Jouf province, Saudi Arabia, during the period September 2008 to May 2009. A total of 930 patients attended the hospital for routine investigation which was screened for urinary tract infection, preliminary identification of the isolates was performed on the basis of colonial morphology, cultural characteristics on agar media, gram's staining reaction and biochemical reaction results using standard methods (Kloos and Bannerman, 1999). *S. aureus* species were identified and methicillin resistant *S. aureus* (MRSA) isolates were detected at the time of initial culture using the disk diffusion method. Antibiotic sensitivity of the isolates initially demonstrating resistance to methicillin was confirmed using BD phoenix TM (System, Becton, Dickinson Company, Shannon, Ireland) according to the recommendations given by the national reference centre in Saudi Arabia. The sensitivity pattern of the isolates was tested for the antibiotics listed in Table 1. Wizard R genomic DNA purification kit+ was used according to manufacturer's instructions (Promega) to isolate the DNA from *S. aureus* clinical isolates.

Amplification of *S. aureus* specific sequence gene and *mecA* gene

PCR was used to amplify both the *S. aureus* specific sequence gene and *mecA* gene with the amplicon size of 107 and 532 bp using primers described by Martineau et al. (1998). The 3-end region of the *S. aureus* specific gene was amplified using A 30 nucleotide forward primer 5'- AAT CTT TGT CGG TAC ACG ATA TTC TTC ACG -3' and A30 nucleotide reverse primer, 5'-CGT AAT GAG ATT TCA GTA GAT AAT ACA ACA-3' (which hybridize to sites 5-34 and (112-83), respectively, (Martineau et al. 1998). While The 3-end region of the *mecA* gene was amplified using A 22 nucleotide forward primer 5'- AAA ATC GAT GGT AAA GGT TGG C - 3' and A22 nucleotide reverse primer, 5'- AGT TCT GCA GTA CCG GAT TTG C-3' (which hybridize to sites 1282-1301 and 1814-1793) (Robert Koch institute, 2003). The PCR reaction mixture for the detection of *mecA* gene and *S. aureus* specific gene consisted of 1 ml of sample containing template DNA. 1.5 u of tag DNA polymerase, 10 µl of 10 x PCR amplification buffer 10 pmol each primer, 200 m/mole deoxynucleotide triphosphate (dNTPS) and distilled water to a final volume of 5010 µl. A total of 40 cycles were used to amplify 532 bp of *mecA* gene and 107 bp of *S. aureus* specific gene. DNA denaturation occur at 94°C for 30 sec primers annealing at 55°C for 30 sec extension of the two strands at 72°C for 60 s and a final extension step of 4 mins. The PCR products were analyzed on a 1.5% agarose gel five micro liters of the PCR products were loaded into 1.5% phorecus agarose (Biogene, UK) and electrophoresis was performed in .5x TBE buffer at 180 V for 3 h. The gels were subsequently stained with 1 µg/ml ethidiumbromide (Sigma, U.K) for 30 min, visualized under UV and photographed.

RESULTS

Nine hundred and thirty urine specimens were received in the laboratory for culture, 640 specimens showed significant growth from which 100 *S. aureus* species were recovered. Using disk diffusion method and BD phoenix TM system antibiotic susceptibility testing identified 15 methicillin resistant *S. aureus* isolates. The antimicrobial agents used their abbreviations potency and origin is shown in Table 1.

All the isolates (n = 100) expressed *S. aureus* specific sequence gene in their PCR products, which confirmed the assumption that all the strains were *S. aureus*. The result of the PCR revealed 13/15 isolates demonstrating both resistance to methicillin and expression of *mecA* gene, while the remaining two showed the resistance to methicillin by the disc diffusion method without the expression of *mecA* gene. All the isolates (n=100) were sensitive to vancomycin.

In Figure 1, all the *S. aureus* isolates were sensitive to vancomycin, while only 2% were sensitive to penicillin and 15% were identified as MRSA. Meropeneme and amoxicillin expressed relatively high activity against the isolates (80 and 74%). Cephalosporin and gentamicin showed the same activity and the least active antibiotic was ciprofloxacin.

According to the Figure 2, all MRSA isolates were sensitive to vancomycin and resistant to penicillin. The isolates expressed high resistant against cephalosporin, gentamicin and ciprofloxacin and relatively low resistant against meropeneme and amoxicillin.

In Figure 3, a 100 molecular weight marker was applied at the first and last well of the gel to identify the isolated genes. A negative control (methicillin susceptible *S. aureus*) PCR product was applied next to the molecular weight marker. (No band on the figer). PCR product of *S. aureus* specific gene 107 bp was applied on lane 3, 5, 7, 9 and 11 which showed a clear band confirmed that, all the isolates were *S. aureus*. PCR product of *mecA* gene 532 bp for the same isolates was applied on lane 4, 6, 8, 10 and 12 which showed clear bands confirmed that, all the isolates were MRSA.

DISCUSSION

Detection of MRSA is important for patient care and appropriate utilization of infection control resources. Methicillin-resistant *S. aureus* (MRSA) is a significant pathogen that has emerged over the last four decades, causing both nosocomial and community-acquired infections. Rapid and accurate detection of methicillin resistance in *S. aureus* is important for the use of appropriate antimicrobial therapy and for the control of nosocomial spread of MRSA strains. We evaluated the efficiency of the disk diffusion method, BD phoenix TM

Table 1. Interpretation of the inhibition zone diameter data.

Antibiotic	Disk potency	Resistant	Sensitive
Penicillin (P)	10 units	<28	> 29
Methicillin (ME)	5 µg	<9	> 14
Gentamycin (Gen)	10 µg	<12	> 15
Ciprofloxacin (CIP)	5 µg	<18	> 21
Meropenem (Mer)	10 µg	<13	> 16
Amoxycillin/Clavulanic Acid (AMX)	30 µg	<17	> 21
Vancomycin (Van)	30 µg	<17	> 21

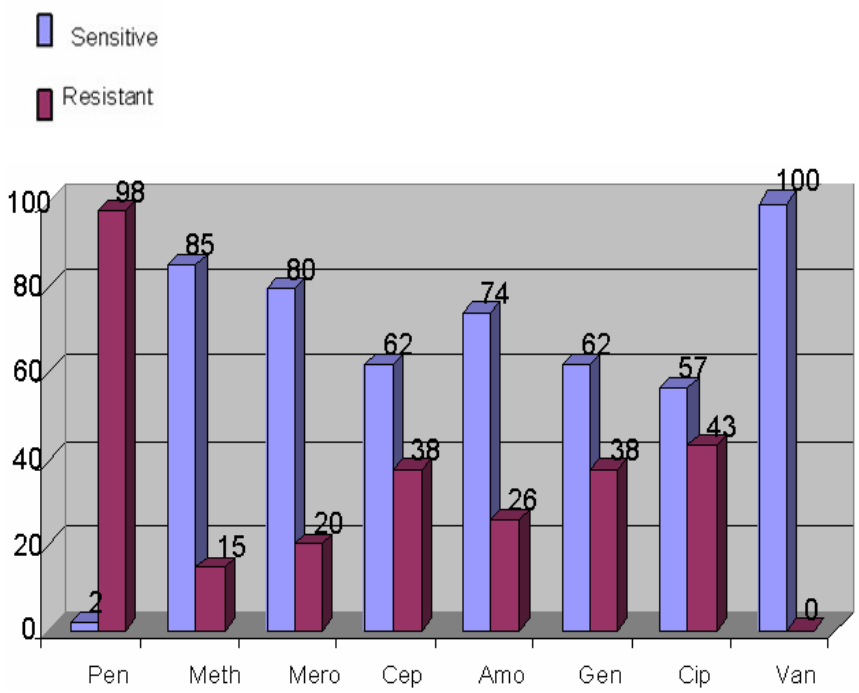

Figure 1. Susceptibility of *S.aureus* isolates to different antibiotics.

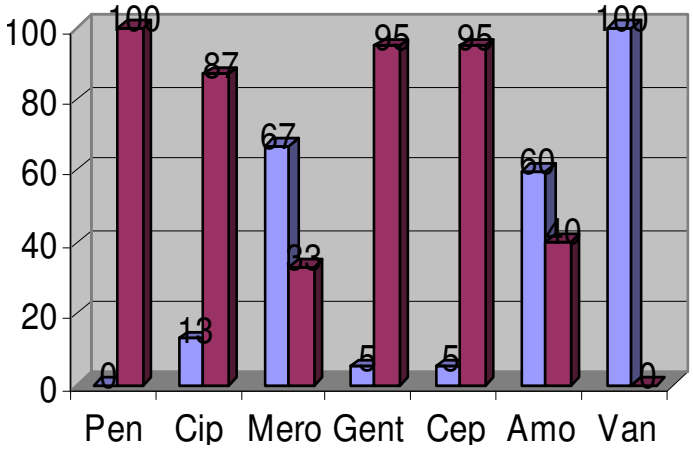

Figure 2. Susceptibility of MRSA isolates to different antibiotics.

system and PCR for detection of methicillin resistance in 100 isolates of *S. aureus*, 13 mecA positive and 87 mecA negative. The PCR of mecA gene was used as the gold standard for the evaluation of the other two methods. The percentages of sensitivity and specificity were as follows; disk diffusion 97 and 100%, and BD phoenix TM system 100 and 100%. The two methods presented high sensitivity and specificity, but BD phoenix had the advantage of giving a reliable result, equivalent to PCR. The incidence of urinary tract infection with *S. aureus* was found to be 15.6%; Beta lactamase producers within the *S. aureus* isolates were 86%, this result agreed with Fukatsu et al. (1990) who reported that, 81.3% of *S. aureus* were beta lactamase producers in Japan. Depending on the methicillin susceptibility testing result15 (15%) of the *S. aureus* isolates were classified as MRSA,

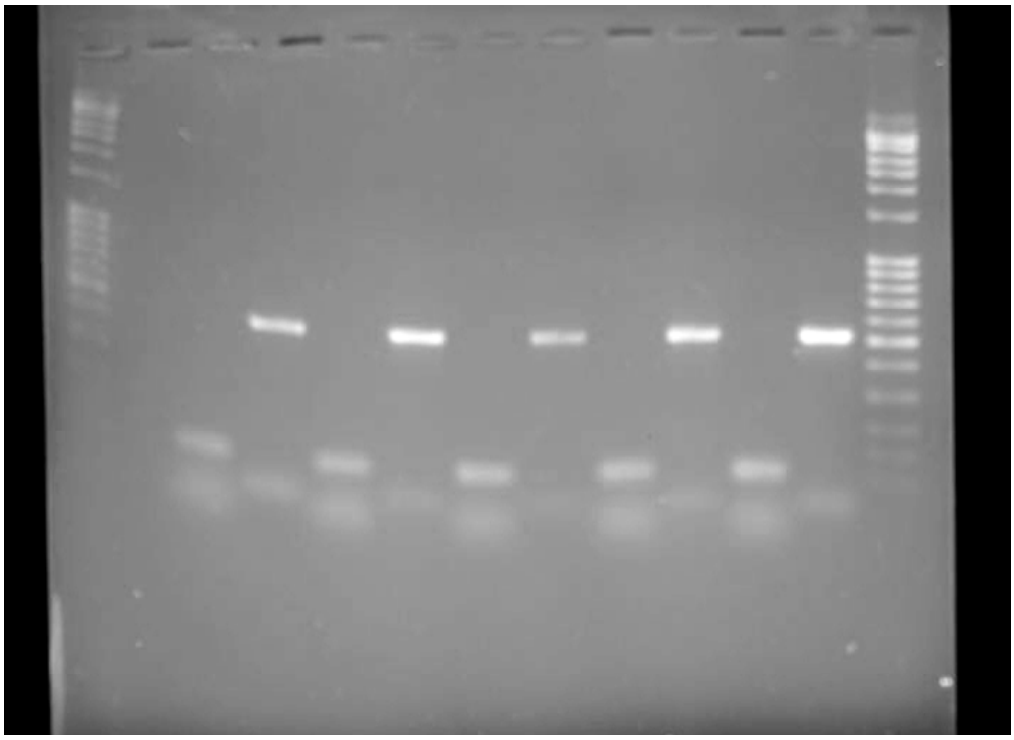

Figure 3. A 100 molecular weight marker applied at the first and last well of the gel to identify the isolated genes. A negative control (methicillin susceptible *S. aureus*) PCR product was applied next to the molecular weight marker. (No band on the figure). PCR product of *S. aureus* specific gene 107 bp was applied on lane 3, 5, 7, 9 and 11 which showed a clear band confirmed that, all the isolates were *S. aureus*. PCR product of *mecA* gene 532 bp for the same isolates was applied on lane 4, 6, 8, 10 and 12 which showed clear bands confirmed that, all the isolates were MRSA.

13/15 (8.7%) of the MRSA isolates expressed *mecA* gene by PCR typing in addition to beta lactamase enzyme production, this result agreed with Tenover et al. (1994) who stated that, the resistance in *S. aureus* mainly involve two mechanisms the expression of beta lactamase and *mecA* gene. In this study, the MRSA resistance pattern was studied against the ten antibiotics excluding penicillin and vancomycin, twelve isolates proved to be resistant to the ten antibiotics, five to eight, three to six and three to four antibiotics. Regarding to the disc diffusion method for the detection of MRSA busy laboratories processing, screening and reading of the specimen results are time-consuming, all isolates are confirmed with tube coagulase and susceptibility testing and keeping agar plates for an extended period (48 h) increases the workup of suspicious colonies significantly, with a small increase in MRSA detection this agreed with Diederen et al. (2006) and Diederen et al. (2005).

In conclusion, molecular techniques remains the most sensitive method in detecting *S. aureus* at both genus and species level and with 100% accuracy in detecting MRSA, when compared with the classical identification method and this agreed with Martineau et al. (2001). In addition, for greater detection rates, molecular methods have the shortest turn around time. Although, molecular testing remains expensive relative to conventional agar-based detection, there is an overall cost savings, especially if molecular testing is directed at high-risk populations.

REFERENCES

Arnold MS, Dempsey JM, Fishman M (2002). The best hospital practices for controlling methicillin resistant *Staphylococcus aureus* on the cutting edge. Infect. Control Hosp. Epidemiol., 23: 69 –76.

Calfee DP, Durbin LJ, Germanson TP (2003). Spread of methicillin-resistant *Staphylococcus aureus* (MRSA) among household contacts of individuals with nosocomially acquired MRSA. Infect. Control Hosp. Epidemiol., 24: 422-426.

Didier G, Stephane B, John S (2004). Amoxicillin-Clavulanate Therapy Increases Childhood Nasal Colonization by Methicillin-Susceptible *Staphylococcus aureus* Strains Producing High Levels of Penicillinase. Antimicrob. Agents Chemother., 48: 4618-4623.

Diederen BML, van Leest, van Duijn I, Willemse P, van Keulen PHJ, J. A. J. Kluytmans W (2006). Performance of MRSA ID, a new chromogenic medium for detection of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 44: 586-588.

Diederen BI, Van Duijn A, Van Belkum P, Willemse P, Van Keulen, Kluytmans J (2005). Performance of CHROMagar MRSA medium for detection of methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 43: 1925-1927.

Fukatsu H, Yamada H, Nonomura H, (1990). Detection of lactamase producing strains isolates form urinary tract and their drug susceptibility, Department of urology, Aichi medical university. Japan.

Girou E, Pujade G, Legrand P (1998). Selective screening of carriers for control of methicillin-resistant *Staphylococcus aureus* (MRSA) in high-risk hospital areas with a high level of endemic MRSA. Clin. Infect Dis., 27: 543 –50.

Harbarth S, Martin Y, Rohner P, Henry N, Auckenthaler R, Pittet D (2000). Effect of delayed infection control measures on a hospital outbreak of methicillin- resistant *Staphylococcus aureus*. J. Hosp. Infect., 46: 43-49.

Hill RA, Duckworth GJ, Casewell M (1988).Elimination of nasal carriage of methicillin resistant *Staphylococcus aureus* with mupirocin during a hospital outbreak. J. Antimicrob. Chemother., 22: 377 –84.

Ibelings MMS, Bruining HA (1998). Methicillin-resistant *Staphylococcus aureus*: acquisition and risk of death in patients in the intensive care unit. Eur. J. Surg., 164: 411 –1.

Jarvis WR, Schlosser J, Chinn RY, Tweeten S, Jackson M (2007). National prevalence of methicillin-resistant *Staphylococcus aureus* in inpatients at US health care facilities, Am. J. Infect. Control., 35: 631-637.

Kearns AM, Seiders J, Wheeler R, Steward M (1999). Rapid detection of methicillin resistant staphylococci by multiplex pCK .J. Hosp. Infect., 43: 33-37.

Kim T, Oh PI, Simor AE (2001). The economic impact of methicillin resistant *Staphylococcus aureus* in Canadian hospitals. Infect. Control Hosp. Epidemiol., 22: 99-104. 13.

Kloos WE, Bannerman TL (1999). *Staphylococcus* and *Micrococcus*. In: Murray PR, ed. Manual of Clinical Microbiology. 7th ed, revised. Washington, DC: ASM Press, 27: 267–269.

Law MR, Gill ON (1998).Hospital acquired infection with methicillin-resistant and methicillin sensitive staphylococci. Epidemiol. Infect., 101: 623 –9.

Lodise TP, McKinnon PS (2005). Clinical and economic impact of methicillin resistance in patients with *Staphylococcus aureus* bacteremia. Diagn. Microbiol. Infect. Dis., 52: 113-122. 14.

Lucet JC, Chevret S, Durand-Zaluski I (2003). Prevalence and risk factors for carriage of methicillin resistant *Staphylococcus aureus* at admission to the intensive care unit. Arch. Int. Med., 163: 181 –8.

Martineau F, Picard FJ, Lansac N (2001). Correlation between the mecA gene determined by multiplex PCR assays and the disc diffusion susceptibility patterns of *Staphylococcus aureus.* Chemother., 44: 231-238.

Martineauf P, Roy PM, Bergeron MG (1998) species-specific and ubiquitous- DNA based assay for rapid identification of *staphylococcus aureus*. Clin. Microbiol., 36: 618-623.

Merrer J, Santoli F, Vecchi C (2000) Colonization pressure and risk of acquisition of methicillin resistant *Staphylococcus aureus* in a medical intensive care unit. Infect. Control. Hosp. Epidemiol., 21: 718 –23.

National Nosocomial Infections Surveillance (2004). Systems Report, data summary from January 1992 through June 2004, issued October 2004 Am. J. Infect. Control., 32: 470-485.

Nimmo GR, Coombs GW, Pearson JC, O'Brien FG, Christiansen KJ (2006). Methicillin-resistant *Staphylococcus aureus* in the Australian community: an evolving epidemic. Med. J. Aust., 184: 384-388. 17.

Pujol M, Pena C, Pallares R (1994). Risk factors for nosocomial bacteraemia due to methicillin-resistant *Staphylococcus aureus*. Eur. J. Clin Microbiol., Infect. Dis. 13: 96 –100.

Rampling A, Wiseman S, Davis L (2001). Evidence that hospital hygiene is important in the control of methicillin resistant *Staphylococcus aureus*. J. Hosp. Infect., 49: 109 –116.

Robert Koch Institute (2003). Multiplex pCR Assay fro simultaneous Detection of Nine clinical Relevant Antibiotic Resistance genes in *Staphylococcus aureus*. J. Clin. Microbiol., 4140: 4089-4094.

Tenover FC, Arbeit R, Biddle J (1994). Comparison of traditional and molecular methods of typing isolates of *Staphylococaus aureus* J. Clin. Microbiol., 32: 407.

Wannet W, Heck M, Pluister G, Spalburg E, De Neeling AJ (2004). Panton-valentine leucocidine positive MRSA in 2003: the Duch situation. Eur. Surveillance 9: 28-29.

Journal of Medical Genetics and Genomics Vol. 3(3), pp. 46 - 49, March 2011
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2011 Academic Journals

Full Length Research Paper

Identification of *Lactobacillus* strains isolated from faecal specimens of babies and human milk colostrum by API 50 CHL system

Dilek Ozgun and Hasibe Cingilli Vural*

Department of Biology, Faculty of Science, Selcuk University, Konya- Turkey.

Accepted 15 Febuary, 2011

Lactic acid bacteria are very significant to human health due to the production of some antimicrobial substances and ability to inhibit pathogenic bacteria. Furthermore, the bacteria are also used as starter culture in various food production. In this study, *Lactobacillus* strains were isolated from 100 human milk colostrum samples and 50 faecal samples of 3 to 30-day old infants who were fed on only breast milk. The isolated *Lactobacillus* strains were futher identified by API 50 CHL systems as *Lactobacillus acidophilus* (20.0%), *L. acidophilus* -3 (10.0%), *Lactobacillus brevis* (30.0%), *Lactobacillus casei* (15.0%,) and *Lactobacillus plantarum* (25.0%) from colostrum, and *L. brevis* (41.2%), *Lactobacillus fermentum* (11.8%), *Lactobacillus reuteri* (5.9%), *Lactobacillus rhamnosus* (11.8%) and *L. plantarum* (29.4%) from faeces. In conclusion, the higher isolation rate of *Lactobacillus* spp. in infantile faecal specimens than in colostrum may indicate based on the sterility of colostrum.

Key words: *Lactobacillus*, MRS agar, API 50 CHL.

INTRODUCTION

The human intestinal tract is inhabited by different species of microorganisms; some of which are responsible for the microbial balance in the normal flora of healthy hosts, since the stability of intestinal system flora is dependent on the interactions between the beneficial and harmful microorganisms (Çakır, 2003). While these beneficial microrganisms like the lactic acid bacteria aid in the digestion of nutritional substances, they also prevent the effects of pathogenic micro-organisms due to certain inhibitory substances which they secret during digestion. These inhibitory substances include metabolites like organic acids, diacetyl, hydrogen-peroxide, acetoin, 2,3-butanediol, acetaldehyde, benzoate, bacteriolytic enzymes,bacteriocin, reuterin, etc (Chung et al., 1989; Vanderbergh, 1993; De Vuyst and Vandamme, 1994) display antagonistic activity towards many pathogenic microorganisms.

The consumption of soured milk can be traced back thousands of years but it was not until the beginning of the 18th century that the beneficial effects of fermented milks were given a scientific basis. Metcnikoff (1907) believed that the microflora of the lower gut was having an adverse effect on the host animal and proposed that fermented milks would reverse the effect and promote good health. He based this hypothesis on observations of Bulgarian peasants who consumed large quantities of soured milk and lived to a ripe old age. Without any real evidence he made the connection between the two observations. Subsequent experiment studies have suggested that there is at least some scientific foundation to the belief that fermented milks have a beneficial effect on the health of the consumer (Fuller, 1991).

Probiotics, are therefore, defined as live microorganisms that confer health benefits to the hosts (Salminen et al., 1993, 1998; Holzapfel et al., 1998, 2001; Madsen, 1999; FAO/WHO, 2001; Perdersen et al., 2004), and some lactic acid bacteria (LAB) species, which include the groups *Lactobacillus*, *Streptococcus*, *Pediococcus* and *Leuconostoc* have been reported as active probiotic candidates by several workers (Fuller, 1991; Ogunshe,

*Corresponding author. E-mail: hcvural@gmail.com.

2008). Fermented Milk Products (FMP) containing viable lactobacilli have been used by humans primarily as prophylactics and their use has been extended to intestinal infections (Ogawa et al., 2001; Ogunshe, 2006, 2008; Ogunshe and Olaomi, 2008). Studies, such as in the treatment of *Clostridium difficile* colitis with *Lactobacillus* have been done on humans (Hopkins and Macfarlane, 2003), while dietary lactobacilli have also been used for the treatment of infantile diarrhoea. Alm (1983), Ogunshe Olaomi (2008) and Ogunshe (2008) observed that the oral administration of *L. acidophilus* reduced the carrier time in children and in adults infected with *Salmonella*.

This study also tries to identify potential indigenous *Lactobacillus* probiotics isolated from 100 human milk colostrum and 50 infantile faecal samples obtained from breast-fed 3 to 30-day-old babies using the API 50 CHL system.

MATERIALS AND METHODS

Materials

This study was carried out between September 2008 and June 2009 in Konya Dr. Faruk Sukan Birth and Childen Hospital and Konya Province Control Laboratory, Turkey. 100 colostrum samples and 50 faecal samples of 3 to 30 day old breast-fed infants were obtained in sterile small jars from Konya Dr. Faruk Sukan Maternity and Children Hospital. Samples were transported within two hours to Konya Province Control Laboratory for microbiological studies.

Media and culture conditions

The colostrum and infantile faecal samples were plated on MRS (Man Rogosa Sharpe) agar supplemented wuth 0.25 % (w/v) L-cysteine (Sigma-Aldrich). One ml of each colostrum and facal samples (after diluting in proportion of 1/10 within MRD (maximum recovery diluent, Merck)) was plated on each MRS agar plate, followed by incubation under anaerobic conditions at 37°C for 48 h using GENbox anaerobic kit (Bio-merieux, Marcy l'Etoile, France). Obtained colonies were randomly picked from the primary plates after incubation and pure colinies identified with API 50 CHL system. Pure colonies were kept as stock and bench cultures in 15% glycerine and preserved in deep- freeze at -20°C.

API 50 CHL system

Fermentation of carbohydrates was determined using API 50 CHL, a standardized system, consisting of 50 biochemical tests for the study of carbohydrate metabolism by microorganisms. API 50 CH is used in conjunction with API 50 CHL medium for the identification of *Lactobacillus* and related genera strips according to the manufacturer's instructions (Biomerieux, Marcy l' Etoile, France) (Ghanbari et al, 2009). 10 ml of pure water was dispensed into the incubation box with the strip placed in the incubation box, after the bacterial cultures had been introduced into the API 50 CHL system in API 50 CHL medium (5 ml), in concentration 2 McFarland. The set-up system was then incubated at appropriate temperature of 35°C for 48 h, after the wells were filled with the bacterial suspensions by the line mark with the addition of mineral oil.

Bacterial strains from colostrum and faecal samples were identified based on the fermentation of carbohydrates. Identification tables were prepared as (+/-) according to colour change in evaluation of results of API strips reaction. Numerical profiles of strains were identified adding positive values in indicative table. Species designations were identified by evaluating with software identification apiweb™.

RESULTS AND DISCUSSION

A total of 20 (25.0%) *Lactobacillus* strains were isolated from 100 colostrum samples in this study. The *Lactobacillus* strains were identified by API 50 CHL systems, program identification apiweb™ as *Lactobacillus brevis* 6 (30.0%), *Lactobacillus plantarum* 5 (25.0%), *Lactobacillus acidophilus* 4 (20.0%), *Lactobacillus casei* 3 (15.0%) and *Lactobacillus acidophilus*-3 in 2 were identified by program identification apiweb™ (10.0%) (Table 1). As shown in Table 2, seventeen *Lactobacillus* strains were also isolated from the 50 infantile faecal samples in this study. They were identified as *Lactobacillus brevis* 7 (41.18 %), *Lactobacillus plantarum* 5 (29.4%), *Lactobacillus fermentum* 2 (11.8%), *Lactobacillus rhamnosus* 2 (11.76 %), *Lactobacillus reuteri* 1 (5.9%). The results obtained in this study also support previous ones in which recovery of similar *Lactobacillus* species from infantile faecal speciemns have been earlier reported (Gronlund et al., 1999; Ahrne et al., 2005).

Lactobacillus strains jist like many other bacterial species have been identified phenotypically with the use of biochemical characteristics and kits like API 50 CH system, and currently by molecular methods, such as with the use of PCR technique. This study identified some *Lactobacillus* strains with the use of the API 50 CH system as belonging to *L. Brevis, L. plantarum*, *L.fermentum*, *L. rhamnosus*, *L. reuteri* from infantile faecal samples. Similar groups of *Lactobacillus* strains had been earlier isolated from infantile faecal specimens from other countries (Apella et al., 1992; Nader de Macias et al, 1992; Ogunshe, 2008), although slight variation which may be due to fields and geographical locations could be observd.

Classically, searching for bacterial strains that are able to exert beneficial effects on human health has been focused on the natural inhabitants of our gastrointestinal tract, mainly being directed at members of the genera *Bifidobacterium* and *Lactobacillus*. In the last few years, a substantial body of scientific evidence suggests that other bacterial genera could rationally be screened for use as probiotics (SÃ¡nchez et al., 2010), thus the screening for likely probiotic *Lactobacillus* strains from human milk colostrum in this study. In contrast to breast milk, little is known about the bacterial composition of human colostrums (Jiménez et al., 2008), although few studies like that of Martín et al. (2010), reported that human breast-milk provides a rich source of commensal lactic acid

Table 1. Recovery rates of *Lactobacillus* strains from colostrum (n = 100) samples.

Species name	Strain number	Strain percentage(%)
Lactobacillus brevis	6	30
Lactobacillus plantarum	5	25
Lactobacillus acidophilus	4	20
Lactobacillus casei	3	15
Lactobacillus acidophilus-3	2	10
Total:5	20	100

Table 2. Recovery rates of *Lactobacillus* strains from faecal (n = 50) samples.

Species name	Strain number	Strain percentage(%)
Lactobacillus brevis	7	41.18
Lactobacillus plantarum	5	29.42
Lactobacillus fermentum	2	11.76
Lactobacillus rhamnosus	2	11.76
Lactobacillus reuteri	1	5.66
Total: 5	17	100

bacteria (LAB) to the infant during breastfeeding and stimulates abundant growth and colonization of these bacteria at mucosal surfaces in the infant gastrointestinal tract. *L. brevis*, *L. plantarum*, *L. acidophilus*, *L.casei* and *L. acidophilus*-3 were the *Lactobacillus* species obtained from the milk colostrum in this study.

It was found that there were no indications that the colostrum samples contained harmful bacteria in the study of Jiménez et al. (2008), while Martín et al. (2010) also documented that breastmilk confers critical nutritional and immunologic support to the developing newborn. The result findings of this present study conclude that beneficial *Lactobacillus* strains can be obtained from colostrum and infantile faecal specimens using API 50 CHL technique.

Acknowledgements

This work was financially supported by Selcuk University Science Research Foundation (Project Grant No: 08201034).

REFERENCES

Ahrne S, Lonnermark E, Wold AE, Aberg N, Hesselmar B, Salman R, Strannegard IL, Molin G, Adlerberth I (2005). Lactobacilli in the intestinal microbiota of Swedish infants. Microbes. Infect., 7: 1256-1262.

Apella MC, Gonzalez SN, Nader de Macias ME, Romero NC, Oliver G (1992). In vitro studies on the inhibition of growth of *Shigella sonnei* by *Lactobacillus casei* and *Lact. acidophilus*. J. Appl. Bacteriol., 73: 480-483.

Alm L (1983). The effect of *Lactobacillus acidophilus* administration upon survival of *Salmonella* in randomly selected human carriers. Prog. Food Nutr. Sci., 7: 13-17.

Chung TC, Axelsson L, Lindgren SE, Dobrogosz WJ (1989). *In vitro* studies on reuterin synthesis by *Lactobacillus reuteri*. Microbial Ecol. Health Dis., 2: 137-144.

Çakır İ (2003). Laktobasillus ve bifidobakterlerde bazı probiyotik özelliklerin belirlenmesi. Ankara Üniv. Fen Bil. Enst., Doktora Tezi, Ankara, 84s.

De Vuyst L, Vandamme EJ (1994). Antimicrobial potential of lactic acid bacteria. *In. Bacteriocins of lactic acid bacteria*. 1st ed. Blackie Academic and Professional, Chapman and Hall, Glasgow. pp.91-142.

Food and Agricultural Organization-World Health Organization (2001). Report of a joint FAO/WHO expert consultants on evaluation of health and nutritional properties of probiotics in food including powder milk with live lactic acid bacteria. Argentina pp.1-4.

Fuller R (1991). Probiotics in human medicine. Gut. 32: 430-442.

Ghanbari M, Rezaei M, Jami M, Nazari RM (2009). Isolation and characterization of *Lactobacillus* species from intestinal contents of *beluga* (*Huso huso*) and Persian sturgeon (*Acipenser persicus*). Iran. J. Vet. Res., 10: 152-157.

Gronlund MM, Lehtonen OP, Eerola E, Kero P (1999). Fecal microflora in healthy infants born by different methods of delivery: permanent changes in intestinal flora after cesarean delivery. J. Pediatr. Gastroenterol. Nutr., 28: 19-25.

Holzapfel WH, Haberer P, Snell J, Schillinger U, Huis in't veid JHJ (1998). Overview of gut flora and products. Int. J. Food Microbio., 41, 85-101.

Holzapfel WH, Haberer P, Geisen R, Bjorkroth J, Schillinger V (2001). Taxonomy and important features of probiotic microorganisms in food and nutrition. Am. J. Clin., Nutr., 73: 3655-3735.

Hopkins MJ, Macfarlane GT (2003). Nondigestible oligosaccaridesenhance bacterial colonization resistance against *Clostridium difficile in vitro*. Appl. Environ. Microbiol., 69: 1922-1927.

Jiménez E, Delgado S, Fernández L, García N, Albújar M, Gómez A, Rodríguez JM (2008). Assessment of the bacterial diversity of human colostrum and screening of staphylococcal and enterococcal

populations for potential virulence factors. Res. Microbiol., 159(9-10): 595-601.
Madsen KL, Doyle LS, Jewll LD, Tavernini MM, Fedorak RN (1999). Lactobacilli species prevent colitis in interleukin 10 gene-deficient mice. Gastroenterol., 116: 1107-1114.
Martín V, Maldonado A, Fernández L, Rodríguez JM, Connor RI (2010). Inhibition of human immunodeficiency virus type 1 by lactic acid bacteria from human breastmilk. Breastfeeding Med., 5(4): 153-158.
Metcnikoff E (1907). Essais optimistes. Paris. The prolongation of life. Optimistic studies. Translated and edited by P. Chalmers Mitchell. London: Heinemann, pp.1900-1904.
Nader de Macias ME, Apella MC, Romero NC, Gonzalez SN, Oliver G (1992). Inhibition of *Shigella sonnei* by *Lactobacillus casei* and *Lact. acidophilus*. J. Appl. Bacteriol., 73: 407-411.
Ogawa M, Schimizu K, Nomoto K, Takahashi M, Watanuki M, Tanaka R, Tanaka T, Hamabata T, Yamasaki S, Takeda Y (2001). Protective effect of *Lactobacillus casei* strain Shirota on Shiga toxin-producing *Escherichia coli* 0157:H7 infection in infant rabbits. Infect. Immunity, 69, 1101-1108.
Ogunshe AAO (2006). *In vitro* bactericidal effects and parental acceptance of indigenous probiotics in the control of infantile bacterial gastroenteritis. Int. J. Probiotics Prebiotics. 1: 233 - 244.
Ogunshe AAO, Olaomi JO (2008). *In-vitro* phenotypic bactericidal effects of indigenous probiotics on bacterial pathogens implicated in infantile bacterial gastroenteritis using Tukey-HSD test. Am. J. Infect. Dis., 4(2): 162-167.
Ogunshe AAO (2008). Bioinhbition of diarrhogenic Gram-positive bacterial pathogens by potential indigenous probiotics in industrial infant weaning food. Asian Pac. J. Trop. Med., 1(2): 7-11.
Perdersen C, Jonsson H, Lindberg JE, Roos S (2004). Microbiological characterization of wet wheat distillers' grain, with focus on isolation of lactobacilli with potential as probiotics. Appl. Environ. Microbiol., 70: 1522-1527.
Salminen S, Deighton M, Gorbach, S (1993). Lactic acid bacteria in health and disease. In *Lactic acid bacteria*. ed. Salminen, S. and von Wright, A. pp. 199-225.
Salminen S, Deighton M, Benno Y, Gorbach S (1998). Lactic acid bacteria in health and disease *In* S. Salmien and A. Won Wright (ed.). Lactic acid bacteria. Microbiology and functional aspects, Marcel Dekker, New York, N.Y. pp. 211-253.
SÃ¡nchez B, Margolles A, Ruas-Madiedo P, De Los Reyes-Gavilan CG, Gueimonde M (2010). Emerging probiotics: the human milk commensal microbiota as an example. Agro Food Ind. Hi-Tech., 21(4): 34-36.
Vanderbergh PA (1993). Lactic acid bacteria, their metabolic products and interference with microbial growth. FEMS Microbiol. Rev., 12: 221-237.

Journal of Medical Genetics and Genomics Vol. 3(3), pp. 50 - 55, March 2011
Available online http://www.academicjournals.org/jmgg
ISSN 2141-2278 ©2011 Academic Journals

Full Length Research Paper

Formulation of nitric oxide donors and antibiotic against typhoid

S. S. Haque

Department of Clinical Biochemistry, Indira Gandhi Institute of Medical Sciences
Patna-14, India. E-mail: sshaq2002@yahoo.co.in.

Accepted 10 February, 2011

Typhoid fever remains a major health problem in India and other developing countries. It often becomes difficult to precisely estimate the global impact of typhoid, as clinical symptoms resemble many other febrile infections. Salmonella, gram negative bacilli, are facultative intra cellular bacteria and can survive during certain stages of host parasite interaction. There are a number of drugs being used for the treatment of typhoid, but increasing occurrence of multidrug resistant (MDR) strain of *Salmonella typhi* has complicated its management; thus, it has necessitated the search of formulated drugs for its treatments. Nitric oxide (NO) is a versatile molecule produced in a biological system, it regulates divers array of physiological function and acts as an inter and extra-cellular messenger in most mammalian organ in host's defense functions for many bacterial infections. Previous studies have suggested that, exogenous administration of L-arginine results in increased NO production, indicating that endogenous substrate is insufficient for maximal NO production. Taking these facts into consideration, it was thought pertinent to see the effect of oral administration of NO donors that is L-arginine, which is one of the semi essential amino acids used as food supplement. Formulation of NO donors and ciprofloxacin are used in low concentrations to reduce toxicity which shows better therapeutic results against experimentally induced Salmonellosis.

Key words: Typhoid, Nitric Oxide and MDR.

INTRODUCTION

Enteric (typhoid) fever remains a common disease in many parts of the world where access to clean water is limited. In places such as India, Nepal, Pakistan, Indonesia and parts of sub-Saharan Africa, typhoid is still a substantial public health problem (Bhan et al., 2005; Crump et al., 2004; Karkey et al., 2008; Parry et al., 2002). Resistance to the traditional first-line antimicrobial agent's ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole defines multidrug resistance (MDR) in *Salmonella enterica.* The MDR phenotype has been shown to be widespread among *Salmonella typhi* for many years (Rowe et al., 1997) and is present, albeit at lower rates, among *Salmonella paratyphi* (Gupta et al., 2008; Parry and Threlfall, 2008). Surveillance studies demonstrate considerable geographic variation in the proportion of *S. typhi* isolates that are MDR in the same region, with sites in India, Pakistan, and Vietnam having higher rates of MDR isolates than sites in China and Indonesia (Ochiai et al., 2008). The wide distribution and high prevalence of MDR among *Salmonella* species has led to fluoroquinolones, assuming a primary role in the therapy for invasive salmonellosis. Some investigators have noted increases in the prevalence of *S. typhi* and *S. paratyphi* strains susceptible to traditional first-line anti-microbials, coinciding with a switch to fluoroquinolones for the management of enteric fever (Maskey et al., 2008; Sood et al., 1999).

Ciprofloxacin continues to be widely used, but clinicians need to be aware that patients infected with Salmonella with decreased ciprofloxacin susceptibility may not respond adequately (Crump et al., 2008). To obtain better understanding of the pathogenesis of typhoid fever, it

seems crucial to elucidate the host defense function of nitric oxide (NO) against Salmonella. NO is a gaseous, inorganic free radical, and produced in biological system. It regulates a diverse array of physiological functions and acts as inter and extra-cellular messenger in most mammalian organs (Misko et al., 1993). Many types of cells, such as leucocytes, hepatocytes, vascular smooth muscle cells and endothelial cells can produce NO during enzymatic conversion of L-arginine to L-citrulline by NO synthetase (NOS). A large amount of NO generated by inducible isoform of the enzyme (iNOS) has been demonstrated. NO functions in biological system in two very important ways. First, it has been found to be a messenger by whom cells communicate with one another (signal transduction). Secondly, it plays critical role in host response to infection. In this second function, it appears that the toxic properties of NO have been harnessed by the immune system to kill or at least slow the growth of invading organisms.

However, excess of NO can exert cytotoxic effects (Stefenovic-Racic et al., 1993). This may involve both i) direct toxicity, e.g., the reaction of NO with iron-containing enzymes of the respiratory cycle and of the DNA synthetic pathway, and ii) the interaction of NO with free radicals like superoxide ion (O_2^-) to form peroynitrite ($ONOO^-$), which is a potent oxidizing molecule capable of eliciting lipid peroxidation and cellular damage (Michael and Feron, 1997). NO is produced in mammalian cells by a group of isoenzymes collectively termed NO synthases (NOS). Nitric oxide has been suggested to inhibit the enzymes such as cyclooxygenase, lipo-oxygenase and cytochrome P_{450} reduction of active site heme or non-heme iron to inactive ferrous form (Kanner et al., 1992). NO has been shown to reduce the ferryl heme by which it prevents many oxidative processes (Gorbunov et al., 1995; Wink et al., 2001).

MATERIALS AND METHODS

Dose and dosage

Animals were divided into six groups. Each group comprised of six animals. The study comprised of the following treatment schedules.

Groups	Treatment
Group 1	Negative control (normal saline)
Group 2	Positive control (*Salmonella typhimurium* ($0.6xLD_{50}$) +Saline
Group 3	*typhimurium* ($0.6xLD_{50}$) +Ciprofloxacin (400 mg per kg b.wt)
Group 4	*S. typhimurium* ($0.6xLD_{50}$) +Arginine (1000mg per Kg b.wt)
Group 5	*S. typhimurium* (0.6xLD50) +Arginine (500mg per kg b.wt)+Ciprofloxacin (200mg per kg b. wt)
Group 5	*S. typhimurium* (0.6xLD50)+Arginine(250mgper kg b. wt) +Ciprofloxacin (200 mg per kg b. wt)

Effects of the aforementioned drugs on infected mice by *S. typhimurium* were analyzed. Post-treatment of drugs were done at the stated dose orally to the experimental animals, the first group was considered as control that received only saline, second group considered as positive control which was challenged with sub lethal dose of *S. typhimurium* ($0.6xLD_{50}$) along with saline. Third group was challenged with sub lethal dose of *S. typhimurium* and given only full dose of ciprofloxacin. Fourth group was challenged with sub lethal dose of *S. typhimurium* and then mice were treated with a full dose of arginine only. The fifth and sixth group animals were challenged with *S. typhimurium* and then half and one fourth dose of arginine was administered along with half dose of Ciprofloxacin respectively. On the 14th day of post treatment, liver was removed aseptically in sterile condition, homogenate was made and post mitochondrial supernatant was prepared for biochemical estimation.

Estimation of lipid peroxidation (LPO)

The modified method of Utley et al. (1967) was used for the estimation of lipid peroxidation. Liver homogenate (1.0 ml) was pipetted in a glass vial of 20 ml and incubated at 37±1°C in a water bath shaker for 60 min at 120 strokes up and down. The other 1.0 ml was pipetted in a centrifuge tube and placed at 0°C and marked as 0 h incubation. After 1 h of incubation, 1.0 ml of 5% TCA and 1.0 ml of 0.67% TBA was added in both samples (that is 0°C and 37°C). The reaction mixture from the vial was transferred to the tube and centrifuged at 1500xrpm for 15 min. The supernatant was transferred to another tube and placed in a boiling water bath for 10 min. Thereafter, the test tubes were cooled and the absorbance of the color was read at 535 nm. The formation of lipid peroxidation was expressed as nmol of malondialdehyde (MDA) formed/h/mg protein.

Reduced glutathione (GSH)

Reduced glutathione in the liver was determined by the same modification in the methods of Jollow et al. (1974). 1.0 ml of PMS (10% w/v) was precipitated with 1.0 ml of sulfosalicylic acid (4%). The samples were kept at 4°C for at least 1 h and then subjected to centrifugation at 1200xg for 15 min at 4°C. The assay mixture contained 0.1 ml of PMS (10% w/v), 2.7 ml phosphate buffer (0.1 M, pH 7.4) and 0.2 ml DTNB (40 mg/10 ml of phosphate buffer, 0.1 M, pH 7.4) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm.

Glutathione peroxidase (GPx) activity

Coupled enzyme assay with glutathione reductase (GR) was used for the estimation of GPX activity. The glutathione disulphide produced as a result of GPX activity, which is immediately reduced by GR thereby, maintaining a constant level of reduced glutathione in a reaction system. The assay takes advantage of concomitant oxidation of NADPH by GR, which was measured at 340 nm.

GPX

$$H_2O_2 + 2GSH \xrightarrow{\text{GPX}} 2H_2O + GSSG$$

GR

$$GSSSG + 2NADPH \xrightarrow{\text{GR}} 2GSH + 2NADP^{+} + H^{+}$$

Specific activity of enzyme was measured according to the procedure described by Mohandas et al. (1984). The reaction mixture consisted of 1.44 ml phosphate buffer (0.05 M, pH 7.0), 0.1 ml of EDTA (1 mM), 0.1 ml of sodium azide (1 mM), 0.1 ml of glutathione (1 mM), and 0.1 ml of NADPH (0.2 mM) ,0.01 ml of hydrogen peroxide (0.25 mM) and 0.1 ml of PMS (10% w/v) in a final volume of 1.95 ml. The enzyme activity was calculated as nmole NADPH oxidized/min/mg protein by using molar extinction coefficient of $6.22 \times 103\ M^{-1}\ cm^{-1}$.

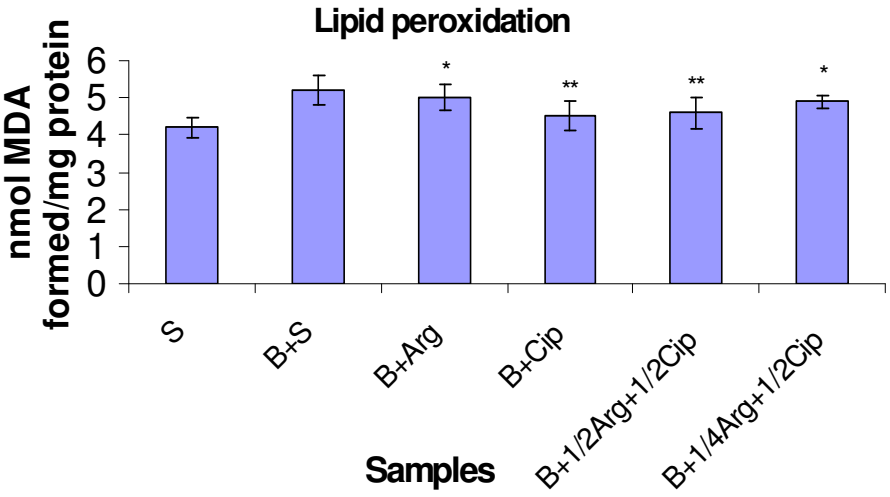

Figure 1. Hepatic malonadialdehyde level measured in mice: drugs were given and study was made at day 14. S = Saline, B + S = *S. typhimurium* + Saline, B + Arg = *S. typhimurium* + 1000mg per kg b. wt L-Arginine, B + Cip = *S. typhimurium* + 400 mg per kg b. wt Ciprofloxacin, B+1/2Arg +1/2Cip=*S. typhimurium* + 500 mg per kg b. wt Arginine + 200 mg per kg b. wt ciprofloxacin, B + ¼ Arg+1/2 Cip = *S. typhimurium* + 250 mg per kg b. wt Arginine + 200 mg per kg b. wt Ciprofloxacin. Values are significantly different *p<0.05 and **p<0.01.

RESULTS

Lipid peroxidation (LPO)

The mice were challenged with a sublethal dose of *S. typhimurium* (0.6xLD_{50}), after seven days, drugs were given up to the next seven days and malonadialdehyde (MDA) production by TBA was measured as an indicator for LPO. The results have been summarized in Figure 1. Infection with *S. typhimurium* significantly induced lipid peroxidation at day 14, as indicated by enhancement of MDA levels compared to the control mice (14.63%). After treatment of L-arginine, Ciprofloxacin and their combination on day 14, it showed 23.40, 25.53, 36.17 and 21.27% decrease in the level of LPO in mice as compared to control. Thus, treatment with L-arginine, Ciprofloxacin and their combination partially protected the liver against the infection-induced damage and some recovery was seen in this dose (B+1/2 Arg+1/2 Cip) in case of lipid peroxidation.

Reduced glutathione (GSH)

To analyze the effect of L-arginine, ciprofloxacin and their combination on liver damage, hepatic GSH levels in mice were measured. The mice were challenged with sublethal dose (0.6xLD_{50}) of *S. typhimurium* and then treated with drugs. The results have been summarized in Figure 2. Infection with bacteria to control mice resulted in significant decrease in the GSH level by 18% at day 14 of infection.

On day 14, the treatment of mice with L-arginine, Ciprofloxacin and their combination, the GSH level was slightly increased by 46.34, 58.53, 53.65 and 46.34% in *S. typhimurium* infected mice as compared with control. Thus, drugs were able to protect the liver from the injury induced by bacterial infection.

Glutathione peroxidase (GPx) activity

To assess the effect of L-arginine, ciprofloxacin and their combination, on liver function. The mice were challenged with a sub lethal dose (0.6xLD_{50}) of *S. typhimurium*, and then treated with the previously stated drugs. GPx activity was assessed and the results have been summarized in Figure 3. Infection of mice with bacteria resulted in a decrease in the GPx activity by 8.63% at day 14 as compared to saline treated control.

On day 14, after the treatment of mice with the previously stated drugs, the GPx activity was significantly increased by 14.54, 15.6, 16.0 and 10.90% as compared with control. However, at day 14, drugs protected the liver, by increasing the decrease of GPx activity and caused to remain near normal to mice.

DISCUSSION

Lipid peroxidation is a chain reaction and a single oxidative event can oxidize many lipid molecules. In the presence of iron or copper ions, the chain reaction can become a cascade and the oxidation process can rapidly

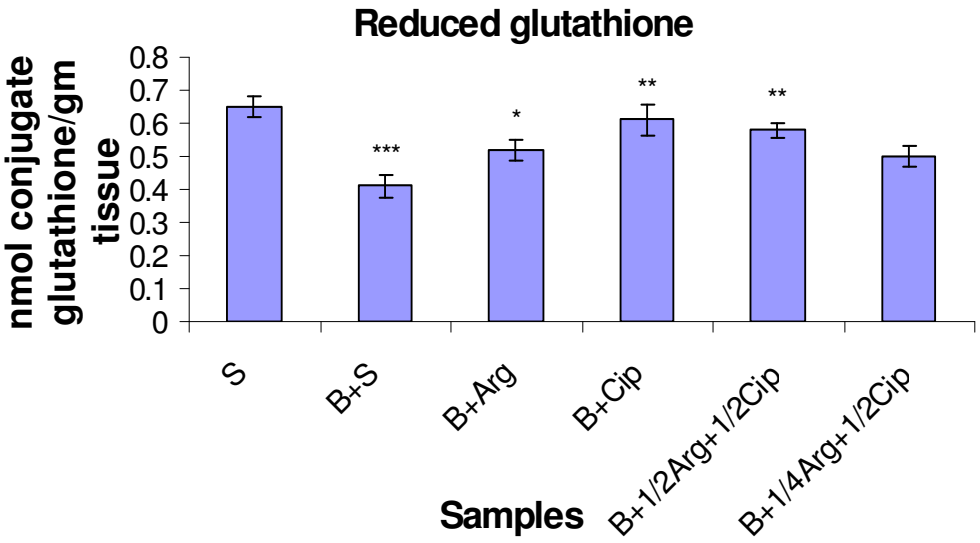

Figure 2. Hepatic reduced glutathione levels in mice: drugs were given and study was made on day 14 with arginine, ciprofloxacin and their combination. S = Saline, B + S = *S. typhimurium* + Saline, B + Arg = *S. typhimurium*+ 1000mg per kg b. wt L-Arginine, B + Cip = *S. typhimurium* + 400 mg per kg b. wt Ciprofloxacin, B + 1/2 Arg + 1/2 Cip = *S. typhimurium* + 500 mg per kg b. wt Arginine + 200 mg per kg b. wt ciprofloxacin, B+1/4Arg+1/2Cip=*S. typhimurium*+250mg per kg b. wt Arginine + 200mg per kg b. wt Ciprofloxacin. Values are significantly different *p<0.05, **p<0.01 and ***p<0.001

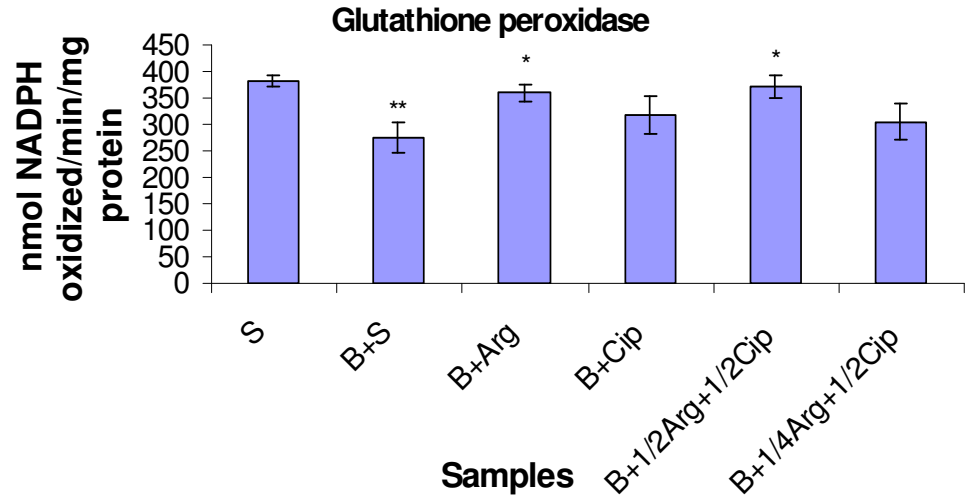

Figure 3. Hepatic GPx activity in mice: drugs were given and study was made on day 14 with arginine, ciprofloxacin and their combination. S = Saline, B + S = *S. typhimurium* + Saline, B + Arg = *S. typhimurium* + 1000mg per kg b. wt L-Arginine, B + Cip = *S. typhimurium* + 400mg per kg b. wt Ciprofloxacin, B + 1/2 Arg + 1/2 Cip = *S. typhimurium* + 500 mg per kg b. wt Arginine + 200 mg per kg b. wt ciprofloxacin, B+1/4Arg+1/2Cip=*S. typhimurium*+250mg per kg b. wt Arginine + 200mg per kg b. wt Ciprofloxacin. Values are significantly different *p<0.05 and **p<0.01.

become unstoppable (Holliwell and Gutteridge, 1984). Nitric oxide reacts with hydroxyl radicals at diffusion-limited rates to generate nitrite, but it clearly cannot exist *in vivo* at a high enough concentration to be an effective scavenger. Nitric oxide has been shown to inhibit the Fenton reaction by binding to ferrous iron and thus, preventing the formation of hydroxyl radicals (Kanner et al., 1991). The most important cellular damage caused by ROS is lipid peroxidation, wherein these reactive oxygen species attack the lipids in the membrane of hepatic cell

and cause peroxidation, resulting in complete destruction of the cell membrane and is associated with Salmonella induced tissue damage. The cell damage was estimated by measuring the changes in the level of lipid peroxidation.

Malondialdehyde (MDA) is a major reactive aldehyde resulting from the peroxidation of biological membranes (Vaca et al., 1988). A secondary product of lipid peroxidation, is used as an indicator of tissue damage involving a series of chain reactions (Ohkawa et al., 1979). Enhancement of the lipid peroxidation in *S. typhimurium* infected mice, as observed in the present study indicates damage to the hepatic cells. It correlates with the change in levels of the serum enzyme also. It has been hypothesized that, one of the principal causes of *S. typhimurium* induced hepatic damage is lipid peroxidation of hepatocytes membrane by generation of free radical derivatives (Recknagel et al., 1991). The observation of elevated levels of hepatic MDA in control mice in the present study is consistent with this hypothesis. In the present study, bacterial infection causes an increase in the LPO levels by 14.63% at day 14 of PI in liver. Drugs showed ability to prevent the *S. typhimurium* induced enhancement of MDA content, suggesting that this combination (B+1/2 Arg+1/2 Cip) inhibit lipid peroxidation more as compared with others (Figure 1).

Reduced glutathione (GSH)

Reduced glutathione (GSH) plays an important role in the detoxification of reactive toxic metabolites of *S. typhimurium*; liver necrosis is initiated when reserves of GSH are markedly depleted (Recknagel et al., 1991; Williams and Burk, 1990). Anand et al. (1996) have suggested that lowered GSH levels may occur due to increased utilization of GSH by antioxidant enzymes. The reduced form of GSH becomes readily oxidized to GSSG on interacting with free radicals. GSH participates in the reductive processes that are essential for the protection of cells through quenching the reactive intermediates and the radicals generated during oxidative toxicity.

Administration of *S. typhimurium* to control mice resulted in reduce levels of GSH. Interestingly, our results showed that L-arginine and ciprofloxacin both were able to enhance GSH levels in infected (B+A and B+C) mice (Figure 2). The maximum increase was found in Ciproflaxacin treated mice at each day 14 of the experiment. These increases in GSH level up regulates NO formation. Infected mice showed a decrease in GSH level, but increase in NO production. The cost for depletion of GSH might be due to the injury mediated by peroxynitrite in host tissues. Peroxynitrite can oxidize GSH to GSSG, which suggests that, this reaction could affect the redox status of intracellular and extracellular thiols (Wink et al., 1997). Thus, in bacterial infected group, the peroxynitrite may deplete GSH by converting it to GSSG. This GSSG may not be recycled back to GSH because there is depletion of glutathione dependent enzymes. The iNOS inhibitor group (IB) showed depletion in GSH level and NO production.

S-nitrosothiols such as *S*- nitrosoglutathione (GSNO) can be formed from NO and reduced thiols (reduced glutahione) in the presence of an electron acceptor (Gow et al., 1997). S-nitrosoglutathione can be bacteriostatic (DeGroote et al., 1995) and bacteriocidal (Shiloh and Nathan, 1997; Chen et al., 2000; Miyamoto et al., 2000). Studies with the Gram-negative bacterium *S. typhimurium* indicate that, S-nitrosoglutathione appears to be recognized as a substrate by the periplasmic enzyme γ-glutamyltranspeptidase, with subsequent conversion to S-nitrosocysteinyl-glycine. This nitrosylated dipeptide in turn is imported into bacterial cytoplasm across the inner membrane by a specialized dipeptide permease (DPP). The presence of dipeptide permease, a member of the ABC (ATP-binding cassette) transporter family, is absolutely essential for GSNO mediated inhibition of *S. tyhphimrium* growth *in vitro*. The ability of GSNO to halt the replication of *S. typhimurium* is markedly reduced by a mutation in DPP, which encodes the dipeptide permease (De Groote et al., 1995).

Glutathione peroxidase

Similarly, our results suggest that enhanced GPx activity was found in liver in combination (B+1/2 Arg+1/2 Cip) of drugs at day 14, maximum increase was seen in L-arginine and combination of drugs (B+1/2 Arg+1/2 Cip) (Figure 3). These results are consistent with report of Farias-Eisner et al. (1996). In contrast to our study, Asahi et al. (1995) on the other hand reported that, GPx could be inhibited by putative NO donor S-nitro-N-acetyl pencillianine (SNAP) in U937 cells. Gluthathione peroxidase can catabolize peroxynitrite *in vitro* (Briviba et al., 1998) and many small biological molecules including glutathione, cysteine, methionine and tyrosine can react with peroxynitrite or its toxic products. Sies et al. (1997) reported a new function for selenoproteins as peroxynitrite reductase. It increased the formation of nitrite from peroxynitrite and was able to defend human fibroblast cells against peroxynitrite mediated oxidation. Peroxynitrite is known to inactivate GPx by the oxidation of essential thiol or selenol (Asahi et al., 1997). Similar trends were observed in case of glutathione reductase activity (Figure 3).

REFERENCES

Anand L, Brajachand NG, Dhanachand CH (1996). Cryptosporidiosis in HIV infection. J. Commun, Dis., 28(4): 241-244.

Bhan MK, Bahl R, Bhatnagar S (2005). Typhoid and paratyphoid fever. Lancet 366(9487): 749-762.

Chen X, Cao J, Xu Q. (2000). Endotoxin exacerbates immunologically induced liver injury in cooperation with interferon-gamma. Inflamm. Res., 49: 571-577.

Crump JA, Kretsinger K, Gay K (2008). Clinical response and outcome

of infection with *Salmonella enterica* serotype Typhi with decreased susceptibility to fluoroquinolones: a United States Food Net multicenter retrospective cohort study. Antimicrob. Agents Chemother., 52: 1278–84.

Crump JA, Luby SP, Mintz ED (2004). The global burden of typhoid fever. Bull. World Health Organ., 82(5): 346-353.

De Groote MA, Granger D, Xu Y, Campbell G, Prince R, Fang FC (1995). Genetic and redox determinants of nitric oxide cytotoxicity in a *Salmonella typhimurium* model. Proc. Natl. Acad. Sci. USA. 92: 6399-6403.

De Groote MA, Granger D, Xu Y, Campbell G, Prince R, Fang FC (1995). Genetic and redox determinants of nitric oxide cytotoxicity in a *Salmonella typhimurium* model. Proc. Natl. Acad. Sci. USA. 92: 6399-6403.

Gow AJ, Thom SR, Brass C, Ischiropoulos H (1997). Electro-chemical detection of nitric oxide in biological systems. Microchem. J. 56, 146-154.

Gupta SK, Medalla F, Omondi MW (2008). Laboratory-based surveillance of paratyphoid fever in the United States: travel and antimicrobial resistance. Clin. Infect. Dis., 46: 1656–1663.

Jollow DJ, Mitchell JR, Zampaglione M, Gillete JR (1974). Bromobenzene induced liver necrosis: Protective role of glutathione and evidence for 3,4-bromobenzene as the hepatotoxic intermediate. Pharmacol. 11: 151-169.

Kanner J, Harel S, Granit R (1991). Nitric oxide as an antioxidant. Arch Biochem Biophys. 289: 130-136.

Karkey A, Aryjal A, Basnyat B, Baker S (2008). Kathmandu, Nepal; Still an Enteric Fever Capital of the World. JIDC, 2(6): 461-465.

Maskey AP, Basnyat B, Thwaites GE, Campbell JI, Farrar JJ, Zimmerman MD (2008). Emerging trends in enteric fever in Nepal: 9124 cases confirmed by blood culture 1993–2003. Trans. R. Soc. Trop. Med. Hyg., 102: 91–95.

Misko TP, Schilling RJ, Salvemini D, Moore W, Currie MG (1993). A fluorometric assay for the measurement of nitrite in biological samples. Anal. Biochem. 214: 11-16.

Miyamoto Y, Akaike T, Alam MS, Inoue K, Hamamoto T, Ikebe N, Yoshitake J, Okamoto T, Maeda H (2000). Novel functions of human alpha(1)-protease inhibitor after S-nitrosylation: inhibition of cysteine protease and antibacterial activity. Biochem. Biophys. Res. Commun., 27; 267(3): 918-23.

Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller D (1984). Differential distribution of glutathione related enzymes in rabbit kidney: possible implications in analgesic neuropathy. Cancer Res. 44: 5086-5091.

Ochiai RL, Acosta CJ, Danovaro-Holliday MC (2008). A study of typhoid fever in five Asian countries: disease burden and implications for control. Bull World Health Organ., 86: 260–268.

Ohkawa H, Ohishi N, Yagi K (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95(2): 351-358.

Parry CM, Hien TT, Dougan G, White NJ, Farrar JJ (2002). Typhoid fever. N. Engl. J. Med., 347(22):1770-1782.

Parry CM, Threlfall EJ (2008). Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. Curr. Opin. Infect. Dis., 21: 531–538.

Recknagel RO, Glende EA, Jr., Dolak JA, Waller RL (1991). Mechanisms of carbon tetrachloride toxicity. Pharmacol. Therap., 43: 139-54.

Rowe B, Ward LR, Threlfall EJ (1997). Multidrug-resistant Salmonella Typhi: a worldwide epidemic. Clin. Infect. Dis., 24: S106–109.

Shiloh M, Ruan J, Nathan C (1997). Evaluation of bacterial survival and phagocyte function with fluorescence-based microplate assay. Infect. Immun., 65: 3193-3197.

Sood S, Kapil A, Das B, Jain Y, Kabra SK (1999). Re-emergence of chloramphenicol-sensitive *Salmonella typhi*. Lancet; 353:

Stefenovic-Racic M, Stadler J, Evans CH (1993). Nitric oxide and arthritis. Arthritis Rheum; 36:1036-44. Michael T, Feron O (1997). Nitric oxide synthesis: Which, where, how and why ? J. Clin. Invest., 100: 2146-2152.

Utley HC, Bernheim F, Hachstein P (1967). Effect of sulfhydryl reagent on peroxidation in microsome. Arch Biochem Biophys., 260: 521-531.

Vaca CE, Wilhelm J, Harms-Ringdahl M (1988). Studies on lipid peroxidation in rat liver nuclei and isolated nuclear membranes. Biochim. Biophys. Acta. 19; 958(3): 375-387.

Williams AT, Burk RF 1990). Carbon tetrachloride hepatotoxicity: an example of free radical-mediated injury. Sem. Liver Dis., 10(4): 279-284.

Wink DA, Cook JA, Kim SY, Vodovotz Y, Pacelli R, Krishna MC, Russo A, Mitchell JB, Jourd'hecil D, Miler AM, Grisham MB (1997). Superoxide moduletes the oxidations and nitrosation of thiols by nitric oxide-derived reactive intermediates: chemical aspects involved in the balances between oxidations and nitrosative stress. J. Biol. Chem., 272: 11147-11151.


Journal of
Medical Genetics and Genomics
Related Journals Published by Academic Journals
■ Journal of Medical Laboratory and Diagnosis
■ Journal of Metabolomics and Systems Biology
■ Journal of Neuroscience and Behavioral Health
■ Journal of Physiology and Pathophysiology
■ Journal of Public Health and Epidemiology
■ Journal of Petroleum Technology and Alternative Fuels
academicJournals