FLUOROQUINOLONE SUSCEPTIBILITY PATTERN OF THE *SALMONELLA* ISOLATES FROM ENTERIC FEVER PATIENTS VISITING TO NATIONAL PUBLIC HEALTH LABORATORY NEPAL

A Dissertation

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In Partial Fulfillment of the Requirements for the Award of the Degree of

Master of Science in Microbiology

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By

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RECOMMENDATION

This is to certify that **Mr. Dhiraj Acharya** has completed this dissertation work entitled **"Fluoroquinolone susceptibility pattern of the *Salmonella* isolates from enteric fever patients visiting to National Public Health Laboratory, Nepal**" as a partial fulfillment of M.Sc. Degree in Microbiology under our supervision. To our knowledge, this is an original research work of his and has not been submitted for any other degree.

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CERTIFICATE OF APPROVAL

On the recommendation of **Dr. Dwij Raj Bhatta** and **Prof. Dr. Sarala Malla**, this dissertation work **by Mr. Dhiraj Acharya** entitled "**Fluoroquinolone susceptibility pattern of the *Salmonella* isolates from enteric fever patients visiting to National Public Health Laboratory, Nepal**" has been approved for the examination and is submitted to the Tribhuvan University for partial fulfillment of the requirement for M. Sc. Degree in Microbiology.

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ABSTRACT

Enteric fever caused by *Salmonella enterica* serovar Typhi and Paratyphi A is the most common clinical diagnosis among febrile patients presenting to hospital in Nepal. The aim of this study was to evaluate the fluoroquinolone susceptibility pattern in *S.* Typhi and *S.* Paratyphi A *Salmonella enterica* serovar Typhi and Paratyphi A. During the study period, 41 isolates of *S. enterica* serovar Typhi (59.54%) and Paratyphi A (41.46%) were grown from 443 blood samples from clinically diagnosed enteric fever patients visiting to NPHL. All isolates were identified by conventional biochemical tests and serotyping with *Salmonella* antisera (Denka Seiken Co. Ltd., Japan). Antibiotic susceptibility testing to 8 antibiotics (Ampicillin, chloramphenicol, cotrimoxazole, tetracycline, nalidixic acid, ciprofloxacin, ofloxacin and ceftriaxone) was performed by Kirby Bauer disc diffusion method and CLSI recommended interpretive criteria. MIC to ciprofloxacin, ofloxacin and nalidixic acid were determined by agar dilution method.

Thirty three (80.49%) isolates were resistant to nalidixic acid, with *S.* Paratyphi A showing higher rate (88.23%) of resistance compared to *S.* Typhi (75%). Two *S.* Typhi isolates (4.88%) were MDR (one showing resistance to ampicillin, chloramphenicol, cotrimoxazole and nalidixic acid and other to cotrimoxazole, tetracycline nalidixic acid, ciprofloxacin, ofloxacin) while none of *S.* Paratyphi A were MDR. The isolates with full resistance to ciprofloxacin and ofloxacin with additional resistance to cotrimoxazole and tetracycline has not previously been characterized from Nepal. Ceftriaxone was the most sensitive (100%) drug. Despite this, one encouraging trend reported is the re-emergence of strains sensitive to ampicillin, chloramphenicol, cotrimoxazole and tetracycline (95.12%).

Difference in both MIC and inhibition zone diameter in nalidixic acid sensitive and nalidixic acid resistant isolates was statistically significant ($P < 0.001$). *S.* Paratyphi A with reduced susceptibility to fluoroquinolones had higher MIC value compared to that of *S.* Typhi ($P < 0.001$). The decreased susceptibility to fluoroquinolones of *S.* Typhi and *S.* Paratyphi A was strongly correlated (sensitivity and specificity of 100%) with resistance to nalidixic acid. Ciprofloxacin and ofloxacin can no longer be considered as the drug of choice in treating enteric fever due to the high level resistant in nalidixic acid and increasing report of full fluoroquinolone resistant. Due to the re-emergence of susceptibility, conventional drug may play important role in the treatment of enteric fever.

Keywords: - Enteric fever, *Salmonella,* Reduced fluoroquinolone susceptibility, Nepal

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ACCo	:	Ampicillin, Chloramphenicol and Cotrimoxazole
ACCoT	:	Ampicillin, Chloramphenicol, Cotrimoxazole and tetracycline
CFU	:	Colony Forming Unit
CLSI	:	Clinical Laboratory Standards Institute
DNA	:	Deoxyribonucleic Acid
ESBL	:	Extended Spectrum Beta Lactamase
FQs	:	Fluoroquinolones
MDR	:	Multi-Drug Resistant
MDRST	:	Multi-Drug Resistant *S.* Typhi
MHA	:	Mueller Hinton Agar
MHB	:	Mueller Hinton Broth
MIC	:	Minimum Inhibitory Concentration
NA	:	Nalidixic Acid
NAR	:	Nalidixic Acid Resistant
NAS	:	Nalidixic Acid Sensitive
NARSPA	:	Nalidixic Acid Resistant *S.* Paratyphi A
NARST	:	Nalidixic Acid Resistant *S.* Typhi
NCCLS	:	National Committee for Clinical Laboratory Standards
NPHL	:	National Public Health Laboratory
PCR	:	Polymerase Chain Reaction
QRDR	:	Quinolone Resistant Determining Region

SD	:	Standard Deviation
SIM	:	Sulfur, Indole, Motility medium
SPA or *S*. Paratyphi A	:	*Salmonella enterica* subspecies *enterica* serovar Paratyphi A
SPIs	:	*Salmonella* Pathogenicity Islands
ST or *S*. Typhi	:	*Salmonella enterica* subspecies *enterica* serovar Typhi
TSI	:	Triple Sugar Iron
T3SS	:	Type III secretion system
WHO	:	World Health Organization