Vol - 4 | Issue - 12 | June - 2015 ISSN No :2231-5063

International Multidisciplinary Research Journal

Golden Research Thoughts

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RNI MAHMUL/2011/38595 **ISSN No.2231-5063**

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International Recognized Double-Blind Peer Reviewed Multidisciplinary Research Journal
Golden Research Thoughts
ISSN 2231-5063 **Impact Factor :3.4052(UIF)**
Volume - 4 | Issue - 12 | June - 2015 Available online at www.aygrt.isrj.org

STUDIES ON PATHOGENS IN ORTHOPEDIC INJURIES IN A TERTIARY CARE HOSPITAL AND PARASITIC INDEX IN SCHOOL CHILDRENACADEMY OF MEDICAL SCIENCES, PARIYARAM, KANNUR, KERALA

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Short Profile

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ABSTRACT:

A study was carried out on the incidence of pathogens associated with wound infections and antibiotic sensitivity of isolates. 50 consecutive wound infected cases from orthopedic department of academy of medical sciences pariyaram, Kannur was taken under study for microbiological examination of bacterial pathogens and antibiotic sensitivity of the isolates was carried. Among this 42 (84%) males and 8 (16%) were females. The pathogen isolated was Staphylococcus aureus, Pseudomonas spp, Citrobacter spp, klebsiella spp, *Escherichia coli, Proteus mirabilis* respectively.Among this *Staphylococcus aureus* was the predominant flora.A single case of Methicillin resistant Staphylococcus was isolated which is considered as hospital acquired.Another study was carried out on the incidence of intestinal parasites in school children of academy of medical sciences pariyaram, Kannur of age group between 3-6.A total of 100 cases constituted the study group.Among this were 60(females) and 40(males).Of this the parasitic incidence was detected only in 3 cases. In one case was the presence of *Entamoeba histolytica*,and in the second case was *Giardia lamblia*, and the third case was *Trichuris trichiura* and Ascaris lumricoides.

KEYWORDS

Parasitic infection, orthopedic infections, isolates .

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INTRODUCTION

Intestinal parasitic infections are the most common cause of health problems in developing countries. It is estimated that at least one quarter of the worlds population is chronically infected with intestinal parasites.(WHO,1996).Of the various infectious parasites,soil transmitted helminth infections are the most common and are an important cause of human morbidity and mortality(*Hall etal,*1992).WHO estimated that Ascaris lumricoides,hook worm and *Trichuris trichiurainfect* 1.4,1.3 and 1 billion people world wide respectively. According to (Chan et al,1994),it is estimated that the global burden of diseases caused by these major intestinal nematodes is 30.0 million disability adjusted life years (DALYs),compared with malaria at 35.7 million and schistosomiasis at 4.5 million. Infection may reach the bones and joints via the blood stream from a distant site, or by direct invasion from a skin puncture, operation or an open fracture. Depending on the type of organism, the site of infection and the host response, the result may be pyogenic osteomyelitis ore arthritis, a chronic granulomatous reaction (classically seen in tuberculosis), or an indolent response to an unusual organism (e.g. a fungal infections) (Salyers, et al, 1994). Thus the aim of the present study was on screening of pathogens in orthopedic injuries in a tertiary care hospital and parasitic index in school children ofacademy of medical sciences, pariyaram, kannur, kerala.

MATERIALS AND METHODS:

a)Incidence of pathogens in would infection from orthopedic department.

Collection of the Sample:

The study population of 50 consecutive wound infected cases from Orthopaedic Dept of Academy of Medical Science.

Sampling Technique

Swabs were prepared by using absorbent cotton on applicator stick. These swabs were kept in a sterile tubes plugged with cotton and sterilized at 1600C for 1 hour in a hot air over. Samples from infected wounds were collected by using the sterilized swabs from the Orthopedic ward. Datas such as Name, Age, Sex, Provisional Diagnosis, Clinical History were noted. The swabs were immediately taken to the Microbiology Laboratory for bacterial identification. The identification of the organisms were upto the level of genus were made in all cases and in few species identification were made. Antibiotic sensitivity tests were done by using Kirby-bauer disc sensitivity method.

Isolation of Bacteria

The pus taken from the infected wound were incubated onto Blood agar and MacConkey agar at 37^0C for 24 hours.

Identification of Bacteria

Identification of bacterial isolates were done based on cultural characteristics, staining techniques, motility and biochemical reactions.

Gram Staining

It was done to differentiate the bacteria into 2 groups, such as gram positive and gram negative. The cultures were smeared on to a clean glass slide, heat fixed and stained with crystal violet and grams iodine respectively for 1 minute. Decolourized with alcohol, counter stained with safranin for 30 seconds and observed under the oil immersion microscope. Those bacteria that appeared violet are reffered as gram positive and those that appeared pink are referred as gram negative.

Biochemical Reactions (IMVIC TESTS)

Indole Tests

This tests determine the ability of an organism to decompose aminoacid tryptophan to indole and nitrogen containing compounds. The test organism is cultured in a medium which contain tryptophan, and incubated at 37^{0}C for 48 hours. After incubation 3-4 drops of kovac's reagent or Ehrlich's reagent (P-dimethylaminobenzaldehyde) was added to culture tubes. Appearance of red ring indicates a positive reaction.

Methyl Red Tests

This tests determines the ability of microorganisams to oxidise glucose with the production and stabilization of high concentration of acid end product. Added a few drops of methyl red solution to the tubes containing broth cultures, which were incubated 370C for 48 hours. Appearance of red colour indicates a positive reaction.

Voges Proskauer Tests

MRVP broth was inoculated with the test culture and incubated for 24 hours. After incubation 0.5 ml of 5% solution of Alpha naphthol in Ethanol and 0.2ml of 40% KOH was added to MRVP cultured broth. Pink colour in 25 minutes and deepening to crimson in 30 minutes indicates VP positive and negative if it remain colourless.

Citrate Utilization Test

This test determines the ability of micro organisms to use citrate as the sole source of carbon. Simmon citrate agar was used the test. Inoculated the simmon citrate agar slants bacterical cultures and incubated at 37^{0}C for 48 hours. Appearance of blue colour indicates a positive reaction.

Triple Sugar Iron Tests(TSI Test)

TheTriple Sugar Iron Tests (TSI Test)is designed to differentiate among the different groups of the family Enterobacteriaceae,according to their ability to ferment lactose,sucrose and glucose and production of H2S.The TSI media is prepared and inoculated with test culture and incubated at 370C for 48 hours .The color change produced in the tube indicates the production of Acid/Alkali respectively. The gas production is indicated by the air bubbles and the H2S production is indicated by the change of medium to black.

Urease Test

This test determines the ability of an organism to degrade urea by the enzyme urease. Inoculated by organisms into the Christensens media and incubated at 370C for 48 hours. Appearance of pink colour indicates a positive reaction.

Catalase Test

This test determine the ability of an organism to degrade H_2O_2 by the enzyme catalase. With the help of inoculation needle or woodenstick the growth from an isolated colony is transferred to a glass slide. A drop of 3% H_2O_2 was added to the bacterial suspension and observed for effervescence. Release of O2 bubbles is positive catalase test.

Oxidase Test

This was done to detect the cytochrome oxidase activity. A single line streak inoculation was done on the agar plates and incubated at 37^0C for 48 hours. Added 2-3 drops of oxidase reagent on the colony and observed the colour change. Colour change from pink purple indicated a positive reaction.

Coagulase Test

This test is used to differentiate *Staphyloccus aureus* (positive) from coagulase-negative Staphyhyocci. *S.aureus* produced two forms of coagulase Bound and free coagulase.Bound coagulase or “clumping factor” is bound to the bacterial cell wall and reacts directly with the precipitates on the staphylococcal cell, causing the cells to clump when a bacterial suspension is mixed with plasma. The presence of bound coagulase correlates with free coagulase, an extracellular protein enzyme that cause the formation of a clot when S.aureus colonies are incubated with plasma. The clotting mechanism involves activation of a plasma (coagulase-reacting factor) which is a modified or derived thrombin molecule, to form a CRF complex. The complex in turn reacts with fibrinogen to produce the fibrin clot.

Slide Test

A colony material is emulsified in 2 drops of saline/sterile H_2O on a glass slide into 2 separate

circle. A drop of plasma Is placed on a suspension in one of the circles and mixed using an applicator stick. Another drop of saline/water is placed on another circle as control. The slide is locked back and forth observing for agglutination of test suspension. A positive reaction is detected within 10 seconds of mixing with the plasma. If no agglutination is seen after 2 minutes, considered as negative. All strains producing negative slide test must be tested with the tube coagulase test.

Tube Coagulase Test

A small amount of a colony growth of the organism is emulsified in a tube containing 0.5ml of plasma. The tube is incubated at 37^0C for 48 hours and observed for clot formation by gently tilting the tube. If no clot is observed, the tube is reincubated and reading is taken after 18 hours.(Ananthanarayan *etal* 2000)

Identification of Streptococcus pyogen

It can be identified by gram staining. It undergoes β −hemolysis and is highly susceptible for "bacitracin" disc on blood agar.

Biochemical Test for the identification of bacteria

Bacteria	Mannitol	Motility	T.S.I.	Peptone	Citrate	Urease
Esch.Coll	+	+	++	+	-	-
Klebsiella	+	-	++	-	+	V
Enterobacter	+	+	++	-	+	V
Citro.diversus	+	+	++	+	+	V
Citro.freundi	+	+	++H_2S	-	+	V
Prot.vulgaris	-	+	-+ H_2S	-	V	+
Morg.morgani	-	+	-+	+	-	+
Prov. rettgerii	+	+	-+	+	+	+
Prov.stuarti	-	+	-+	+	V	V
Prov.alklificans	-	+	-+	+	V	-
Salm.typhi	+	+	-+ H_2S	-	-	-
Salm.para.A	+	+	-+	-	-	-
Other Salmonella	+	+	$-_+$ H_2S	-	+	-
Pseudo.aeroginosa	-	+	--	-	+	V
Non-fermenting GNB	-	-	--	-	V	V
Vibrio cholera	+	+	++	+	-	-
V-variable	M	M	T	P	C	u

Antibiotic sensitivity or bacterial isolates

Organisms isolated from the samples were subjected to antibiotic sensitivity test. Susceptibility test was done using Kirby-Bauer Disc Diffusion technique on Muller Hinton Agar (P^H 7.4 – 7.5)(PLATE 1)

Preparation of Antibiotic Disc

Commercially prepared (Hi Media Laboratory Disc) antibiotic disc were used.

MHA plates were prepared and inoculated with isolated organisms. Using sterile cotton swabs, sterile discs containing antibiotics were applied on the plates incubated at 12-18 hours and observed for zone formed were measured and expressed in millimeters.

ANTIBIOTICS	SYSMBOL
AMPICILLIN	A
AMIKACIN	AK
ERYTHROMYCIN	E
GENTAMICIN	G
NETILMYCIN	Nt
CIPROFLOXACIN	Cf
COTRIMOXAZOLE	Co
CEPHOTAXIME	Ce
CEFOPERAZONE	Cs
CEFUROXIME	Cu
PENCILLIN	P
BACITRACIN	B

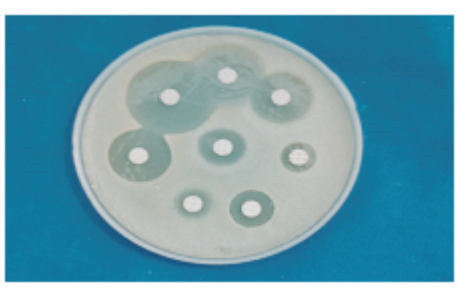

Plate 1 Antibiotic Sensitivity of Gram Negative Bacilli (E.coli, Klebsiella)

Incidence of intestinal parasites among school children of ACME,public school pariyaram Sample collection and fecal examination .A total of 100 cases constituted the study.Among this 60(females) and 40(males) of the age group between 3-6.Prior to commencing the survey, we explained our purpose to the children, their parents, and teachers. We distributed a plastic container for fecal collection to the children who showed understanding of our purpose and offered cooperation

in obtaining samples. Fecal samples were suspended in more than five volumes of MIF (merthiolate, iodine, and formalin) fixative solution, mixed thoroughly, and stored at room temperature. About 3ml of fecal suspension, equivalent to 0.5g of feces, was examined for helminth eggs using the formalin-either sedimentation technique (FES). Twenty microliters of the fecal sediment was used for microscopic observation (x100) and all eggs observed in the wet mount were counted if the sample was positive.*Cryptosporidium parvum* and *Cyclospora* sp were examined using primarily the sucrose floatation technique(SFL).Briefly a sucrose solution with a specific gravity of 1.200 was added to the .5 g of fecal sediment ,mixed thoroughly ,and centrifuged at 1,100 g for 10 minutes.The surface solution was recovered using a bacteriological loop and observed under the microscope. (Arora *etal*,1995)

Questionnaire Survey

To investigate the relationship between parasitic infection and the children's life styles, a questionnaire survey was conducted. The questionnaire included 10 questions regarding the children's body height, weight, and school records; and parents educational background, income, and occupation.

RESULT AND DISCUSSION

The present study was carried out on the incidence of pathogens associated with wound infections and antibiotic sensitivity of isolates. 50 consecutive wound infected cases from orthopedic department of academy of medical sciences pariyaram, Kannur,kerala was taken under study for microbiological examination of bacterial pathogens and antibiotic sensitivity of the isolates was carried. Among this 42 (84%) males and 8 (16%) were females. The pathogen isolated was *Staphylococcus aureus*, Pseudomonas spp, Citrobacter spp, klebsiella spp, *Escherichia coli, Proteus mirabilis* respectively. Among this *Staphylococcus aureu*s was the predominant flora. A single case of Methicillin resistant *Staphylococcus* was isolated which is considered as hospital acquired.A rare case report of *Vibrio metschnikovii* was reported by (Hans etal2004) on a post operative wound infection on thelower right leg of a patient after *saphenectomy.* Acase report of sternal osteomyelitis caused by Staphylococcus schleiferi was reported by(Jorge etal 2000).Another case report was reported by (Chandra etal 2000,)Salmonella osteomyelitis,acase of complication of typhoid fever.(Lazzarini *etal*2004) reported a case report of Clostridial orthopaedic patients isolated from soil and intestinal tract of humans.

Another study was carried out on the incidence of intestinal parasites in school children of academy of medical sciences pariyaram, Kannur of age group between 3-6.A total of 100 cases constituted the study group.Among this were 60(females) and 40(males).Of this the parasitic incidence was detected only in 3 cases. In one case was the presence of protozoan parasites *Entamoeba histolytica,*and in the second case was *Giardia lamblia*, and the third case was of helminthic parasites *Trichuris trichiura* and Ascaris lumricoides.(Srijan *etal*,2007) conducted a study on intestinal parasites in preschool children Thailand and reported the the presence of *G.lamblia* and *Cryptosporidium* spp.Another study was made by (Choomanee *etal*,2007) on the intestinal parasitic infection among school children in Thailand and reported the prevalence of protozoan infections was significantly higher than helminth infection.(Uga *etal* 2005) studied the intestinal parasitic infection in school

children in a suburban area of Vietnam reported the the presence of helminthic and protozoansparasites which includes *Entamoeba histolytica.Entamoeba .coli,Giardia lambliaTrichuris trichiura* and Ascaris lumricoides etc .

The school based approach is one the range of strategies that can be used for helminthic control at community level ;it can be integrated into existing system such as maternal and child health care, family planning, water supply and sanitation should be reinforced through health education. (subash,1995)

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