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ANTIBACTERIAL ACTIVITY OF HUMAN AMNIOTIC FLUID

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ABSTRACT
The present study investigated that the antibacterial activity of human amniotic fluid against some common bacterial pathogens. The common bacteria studied were *E. coli. Proteus mirabilis* and *Klebsiella pneumoniae*. The amniotic fluid was collected through a thin syringe from pregnant women during her parturition stage while the caesarean section delivery. The antibacterial activity was performed by cup well method. The amniotic fluid showed good inhibitory activity against all tested bacteria. The most susceptible bacteria were found to be *Proteus mirabilis* showed 25 mm zone of inhibition. *E. coli* showed maximum zone of inhibition of 20mm whereas *K. pneumoniae* showed 15 mm of zone of inhibition. This finding clearly demonstrated the amniotic fluid showed good inhibitory activity against common bacterial pathogens.

KEYWORDS: Antibacterial activity, Amniotic Fluid.

INTRODUCTION
Amniotic fluid (AF) is a complex and dynamic milieu that changes as pregnancy proceeds. Amniotic fluid contains nutrients and growth factors which are essential for fetal growth provides mechanical cushioning and antimicrobial effectors that protects the fetus and allow assessment of fetal maturity and disease. During embryogenesis, AF volume increases faster than embryonic size. The water in AF originally comes from maternal plasma and passes through the fetal membranes based on hydrostatic and osmotic forces. As the placenta and fetal vessels develop, water and solute from maternal plasma pass across the placenta to the fetus and then to the AF. In the early fetal period, AF volume and fetal size are related in a linear fashion. AF volume increases from about 25 ml at 10 weeks to about 400 ml at 20 weeks. During this period, AF composition is similar to fetal plasma. There is rapid bi-directional diffusion between the fetus and the AF across the not-yet-keratinized fetal skin and the surfaces of the amnion, placenta and umbilical cord, each being freely permeable to water and solutes. During this phase of pregnancy, the AF serves both as a physiologic buffer and an extension of the fetal extracellular compartment. By 8 weeks of gestation, the urethra is patent and the fetal kidneys make urine. Shortly thereafter fetal swallowing begins; however, neither fetal urination nor swallowing contributes significantly to the content or volume of AF until the second half of pregnancy. Keratinization of fetal skin begins at 19 to 20 weeks of gestation and is usually complete at 25 weeks after conception. When keratinization is complete, the relationship between fetal size and AF volume is no longer linear. By 28 weeks of gestation, AF volume reaches a volume of 800 ml where it plateaus near term gestation and thereafter declines to B400 ml at 42 weeks (Mark A. et. al., 2005).

In general, amniotic fluid (AF) has normally been considered sterile. AF contains several humoral and ceḷḷuḷar antimicrobial factors and its antibacterial activity increases during pregnancy. The presence of microorganisms in AF has usually been associated with infection, which may result in spontaneous abortion, pre-term delivery, fetal and neonataḷ infections or postpartum endometritis. However, asymptomatic women with intact membranes may also sometimes (incidence 1.4-24%) have microorganisms in their AF, as shown by several studies. Recent data suggest that this value is even higher when modern methods, e.g. polymerase chain reaction (PCR), are used to detect microorganisms. It has been demonstrated that some microbes, e.g. group B *streptococci* (GBS), *Candida* spp., *Staphylococcus epidermidis* and Ureaplasma urealyticum are able to cross intact membranes. Thus, a simple positive smear or cuḷture of AF may indicate not the presence of infection but the translocation of microorganisms from vaginal or cervical microflora (Reet Mandar et. al., 2001).

Composition of Amniotic Fluid
Amniotic fluid consist of 98% to 99% water. Early in human pregnancy the AF is isotonic with maternal or fetal plasma and contains minimal amount of proteins. In early gestation, substantial amount of amniotic fluid are present before the establishment of fetal urine production. Little is known about the dynamics of amniotic fluid early in gestation, but a likely scenario is

the active transport of solutes across the amnion into the amniotic space with water moving passively down the chemical gradient. Fluid may also arise as a transudate of plasma across fetal non-keratinized skin or from the mother across the uterine deciduas and\or the placental surface.

Much more is known about the dynamics of the AFV in the second half of the pregnancy after fetal skin keratinizes at 22nd to 25th weeks of gestation, resulting in the prevention of further water movement across the skin. As the gestational age increases, amniotic fluid osmolality and sodium concentration decreases, which is thought to be due to the increasing production of diluted featal urine.AF osmolality ultimately reaches to 250-260 mOsm/ml at term. Fetal osmolality (~278mOsm/ml) remains close to maternal osmolality (280mOsm/ ml), which limits the amount of water transfer between the fetal and maternal circulation under normal condition (Xin Wang et. al., 2012).

Protective Antimicrobial Role of Amniotic Fluid

AF plays an important protective role by providing a supportive cushion allowing fetal movement and growth. The oligohydramnios sequence and its related fetal deformations demonstrate the importance of this protective cushion. AF also has a significant defensive role as a part of the innate immune system. The innate immune system is the first line of defense against pathogens and includes anatomic and physiologic barriers, enzymes and antimicrobial peptides, as well as phagocytosis and release of proinflammatory mediators by neutrophils and macrophages. Many of the substances that comprise the innate immune system have been identified in AF and vernix and have been shown to have significant antimicrobial properties; these include the a-defensins [HNP1-3], lactoferrin, lysozyme, bactericidal/permeability-increasing protein, calprotectin, secretory leukocyte protease inhibitor, psoriasin and a cathelicidin These potent antimicrobials show broadspectrum activity against bacteria, fungi, protozoa and viruses. Perhaps the most important of these are the a-defensins which are found in significant concentrations in AF of women without evidence of infection and likely originate from the fetal skin and lung. AF concentrations of HNP1-3 increase with preterm labor, preterm premature rupture of membranes and chorioamnionitis probably due to release from neutrophils (Akinbi HT et. al., 2003; Yoshio H et. al., 2004).

Lactoferrin (LF) is a glycoprotein with two binding sites for ferric ion. LF is found in human milk and appears in human AF at 20 weeks gestation increasing in concentration with gestation. Elevated levels of LF have been noted with preterm labor and with amnionitis. In pregnancies complicated by intra-amniotic infection (IAF), LF is likely secreted by neutrophils in the AF and by amniotic cells. LF has both bacteriostatic activity, due to sequestration of iron which is then unavailable for microbial growth and bacteriocidal activity, due to binding to bacterial outer membranes triggering release of lipopolysaccharide. Enzymatic digestion of LF at acid pH releases a potent cationic, microbicidal peptide called lactoferricin. Lactoferricin shows antimicrobial effects against viruses, protozoa and fungi. Lactoferrin levels decrease with the onset of term labor. The activity of the ‘‘cellular’’ innate immune system within AF as a protective mechanism for the fetus is less well defined. The numbers of mononuclear phagocytes (i.e., monocytes, macrophages, histiocytes) in AF are limited in normal pregnancies, while their numbers are increased in fetuses with neural tube defects. Whether these macrophages are present to prevent infection because of a disruption of the fetal skin or as scavenger cells to clean up neural debris is uncertain. Neutrophils are not normally identified in the AF of healthy fetuses, but are useful as a marker of AF infection. These cells are fetal in origin and appear to originate in the fetal vessels of the chorionic plate. It is interesting that meconium stained AF shows chemotactic activity for neutrophils in utero, although the meconium itself is not the likely chemotactic factor. Two hematopoietic growth factors, G-CSF and macrophage colony-stimulating factor (M-CSF), are found in AF of healthy term and preterm fetuses. G-CSF is elevated in the serum of women with subclinical chorioamnionitis, in the cord blood of neonates with infection, fetal distress, premature rupture of membranes and meconium staining of AF and in the AF, neonatal urine and neonatal bronchoalveolar fluid of newborns after IAI. Whether G-CSF and M-CSF actually play a preventive host defense role in the AF or are just excreted by-products of the immune response during infection is not known (Otsuki K et.al., 1999).

MATERIALS AND METHODS

Collection of Fluid

Amniotic fluid was collected through a thin syringe from a pregnant women during her parturition stage while the caesarean section delivery. The gynecologist pierced the needle the needle and punctured the amniotic sac and injected out an amount of amniotic fluid into a sterilized glass container and the glass container was properly packed. In the same way four amniotic fluid samples were collected from different pregnant women. Some of the amniotic fluid samples were blood stained and some had meconium residue. Such samples were allowed to settle down and was separated using micropipette.

Collection of microbial pathogens

Pathogenic bacteria such as *Proteus mirabilis, Escherichia coli* and *Klebsiella pneumoniae* were obtained Medical lab of P.G. Department of Microbiology, RTM Nagpur University, Nagpur and were maintained on Nutrient agar slant for further use at 4^0C.

Antimicrobial activity of amniotic fluid crude

Amniotic fluid is collected from the maternity hospital and is stored in incubator at 37^0C. (do not refrigerate). The sterile nutrient broth was inoculated with test

organism and incubate at 37^0C for 6 hours old culture. Muller Hinton agar was sterilized by autoclaving and poured into petriplate 20ml each and then was allowed to solidify. Dip a sterile non-toxic cotton swab into six hours old broth culture of test organism and streak the soaked swab firmly on the agar surface. Allow the swabbed organism to dry for 5 min. With the help of alcohol sterile cork borer (7mm in diameter) wells were cut into respective petriplates. 100µl of amniotic fluid (crude) were added in two different wells. And the 3rd well was filled with sterile distilled water as control. The plates were incubated at 37^0C for 24 hours. The diameter of zone of inhibition in millimeter (mm) was measured using zone reader.

RESULTS AND DISCUSSION

Human embryonic membrane consists of chorion, allantois and amnion. Among that, amniotic membrane is the inner most layer of the three constituent layers of the fetal membranes (M. Parthasarathy et. al., 2010; Barachetti, L et. al., 2014). The antimicrobial properties of amniotic fluid may be due to the presence of lysozymes, 7S immunoglobulin and IgA (aa). Antimicrobial properties are well described for amniotic fluid but a limited number of studies only have pointed human fetal membranes, especially the amniotic and chorionic membranes (Burleson R. et. al., 1972). Nowadays, there is an emerging need of membranes in tissue engineering and it has been applied therapeutically to ulcerated skin surfaces, peritoneum and the lacerated eye (Colocho G. et. al., 1974; Trelford J.D. et. al., 1979) microbial infections (Barequet I.S et. al., 2008). So, in recent years the studies have focused on human fetal membranes to check the repairing ability and its use in reconstructions after microsurgery and keratectomy (Soltan, M. et. al., 2013). Four samples of Amniotic fluid crude and supernatant were used for demonstrating the antimicrobaial effect of both the forms respectively. *E.coli* gave zone of inhibition against amniotic fluid crude as well as suppernatant. the zone of inhibition of four samples of crude amniotic fluid given by *E.coli* was 16mm, 11mm, 20mm, 18mm respectively. And in case of supernatant the two supernatant sample of amniotic fluid gave zone of measure 20mm and 18mm. *P.mirabilis* showed zone of inhibition against amniotic fluid crude as well as suppernatant. the zone of inhibition of four samples of crude amniotic fluid given by *E.coli* was 20mm, 22mm, 24mm, 25mm respectively. And in case of supernatant the two supernatant sample of amniotic fluid gave zone of measure 31mm and 22mm. *Klebsialla pneumoniae* was found to be showing zone of inhibition only against amniotic fluid supernatant producing the zone of measure 19mm and 23mm respectively. Out of two samples, such as crude and supernatant, supernant sample of amniotic fluid showed good inhibitory activity compared to crude sample. This study showed that the aminiotic fluid having good antibacterial activity against tested bacteria. Now a days, irrational use of antibiotics in humans and animal species, insufficient patient education when antibiotics are prescribed, lack of guidelines for treatment and control of infections, inadequate dissemination of scientific information for physicians on the rational use of antibiotics and lack of official government policy on the rational use of antibiotics in public and private hospitals, have all contributed to antibiotic resistance (Xin Wang et. al. 2013). Therefore, there is a need to develop alternative antimicrobial therapy for the treatment of infectious diseases from aminiotic fluid.

CONCLUSION

The present study clearly confirmed the antibacterial effect of amniotic fluid against several bacterial pathogens. Amniotic fluid can be used as an alternative drug used against resistance bacteria.

Table No. 1: Antibacterial activity of amniotic fluid against bacterial pathogens.

Sr. No.	Amnioitc fluid	*E. coli*	*P. mirabilis*	*K. pneumoniae*
1	Sample 1 (Crude)	16mm	20mm	12mm
2	Sample 2 (Crude)	11mm	22mm	14mm
3	Sample no. 3 (Supernatant)	20mm	24mm	15mm
4	Sample no.4 (Supernatant)	18mm	25mm	14mm

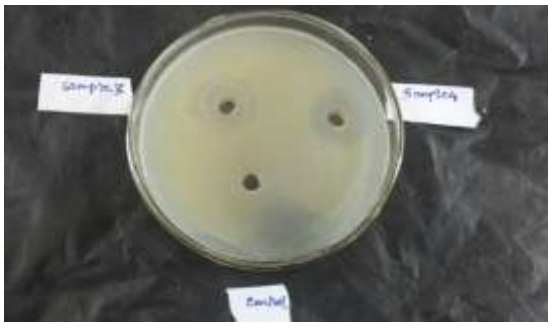

Fig 1: Antibacterial activity of aminiotic fluid against *Proteus spp.*

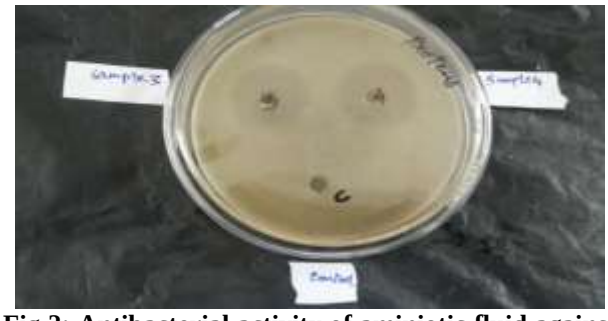

Fig 2: Antibacterial activity of aminiotic fluid against *E.coli*

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