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RESEARCH ARTICLE

Antimicrobial Activity of Human Amniotic and Chorionic Membranes

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Abstract

The present study investigated the antimicrobial activity of human amniotic and chorionic membranes against some common bacterial and fungal pathogens. The findings clearly demonstrated the antimicrobial effect of both the amniotic and chorionic membranes against the tested bacterial and fungal pathogens at different dilutions by their maximum and minimum inhibitory zones. The maximum inhibition zone was measured in amniotic membrane compared to chorionic membrane in both the bacterial and fungal activity plates. While assessing the four different dilutions ($5X10^5$, $5X10^6$, $5X10^7$ and $5X10^8$), the similar diameter of inhibition zone was observed in $1.5X10^5$ and $1.5X10^6$ dilutions. The study clearly confirmed the antimicrobial activity effect of both amniotic and chorionic membranes against several bacterial and fungal pathogens in which maximum activity was recorded by amniotic membrane.

Keywords: Antimicrobial activity, human amniotic membrane, chorionic membrane, inhibition zone.

Introduction

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 (Barachetti *et al*., 2010). The fetal membrane and transparent membrane are composed of an inner epithelial layer that is laid on the basement membrane which in turn is connected to a thin membrane of connective tissue through thin filaments comprising of interstitial collagen I, III and V. Generally the epithelial layer is cube-shaped mononuclear cells with some cytoplasmic vacuoles and the basement membrane is thin contains a fibrous mesh network (John, 2003). The connective tissues are mainly composed of three layers namely compressed, fibroblast and spongy layer. The amniotic membrane thickness is 0.02-0.5 mm covers the amniotic cavity and its apical interior surface is in contact with amniotic fluid while the external surface is in direct contact with the chorionic membrane (Toda *et al*., 2007). In general, embryonic membrane protects the embryo from the microbial infections (Barequet *et al*., 2008). The membrane of amnion and chorion are important sources of natural antimicrobials found in the uterus and they are produced in amniotic fluid during pregnancy and localize in the placenta, uterus endometrium and fetal membranes (Stock *et al.,* 2007). 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 and Eiseman, 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 *et al*., 1974; Trelford and Sauder, 1979).

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 *et al*., 2013). Against these backdrops, this study was aimed to check the antimicrobial activity of human amniotic and chorionic membranes against different bacterial and fungal pathogens.

Materials and methods

Collection of membrane: After the delivery of pregnant women, the human fetal membrane (placenta) was collected and transferred using vaccine carrier (which maintains the temperature below -4°C) to the laboratory within 1 h. Then to remove blood clots, they were washed with saline water under sterile condition and the inner amniotic membrane was separated from the chorion through blunt dissection. Amniotic membrane was washed three times with phosphate buffer saline (PBS) containing the antibiotics cloxacillin (50 µg/mL), streptomycin (50 µg/mL) and amphotericin B (2.5 µg/mL) to remove the microbial agents (Mahon and Manuselis, 2007).

Collection of microbial pathogens: Pathogenic bacteria such as *Staphylococcus aureus, Proteus mirabilis, P. vulgaris, Salmonella* sp*., Pseudomonas aeruginosa, Escherichia coli*, *Vibrio cholerae*, *V. parahaemolyticus*, *Shigella* sp. and fungi such as *Aspergillus niger, A. flavus, A. nidulans* and *A. fumigatus* were obtained from Dept. of Biotechnology, Vel Sri Ranga Sanku Arts and Science college, Avadi, Chennai, Tamil Nadu, India and were maintained in specific broth and agar medium.

Table 1. Antibacterial activity of human amniotic and chorionic membranes against different pathogenic bacteria at various dilutions.

Bacterial pathogens	Amniotic membrane				Chorionic membrane			
	$5X10^5$	$5X10^6$	$5X10^7$	$5X10^8$	$5X10^5$	$5X10^6$	$5X10^7$	$5X10^8$
Staphylococcus aureus	+++	+++	+++	+++	+++	+++	+++	++
Proteus mirabilis	++	+++	++	+	++	++	+	+
Proteus vulgaris	+++	+++	++	+	+++	+++	+	+
Salmonella sp.	+++	+++	++	++	++	++	+	+
Pseudomonas aeruginosa	++	++	++	+	++	++	++	+
Escherichia coli	+++	+++	+++	+++	+++	+++	+++	+++
Vibrio cholerae	+++	+++	++	++	+++	+++	+	+
Vibrio parahaemolyticus	+++	+++	++	+	+++	+++	+	+
Shigella sp.	+++	+++	+++	+++	+++	+++	++	++

- : No inhibition zone, + : ZOI up to 4 mm, ++ : ZOI up to 8 mm, +++ : ZOI up to 12 mm.

Table 2. Antifungal activity of human amniotic and chorionic membranes against different fungi at various dilutions.

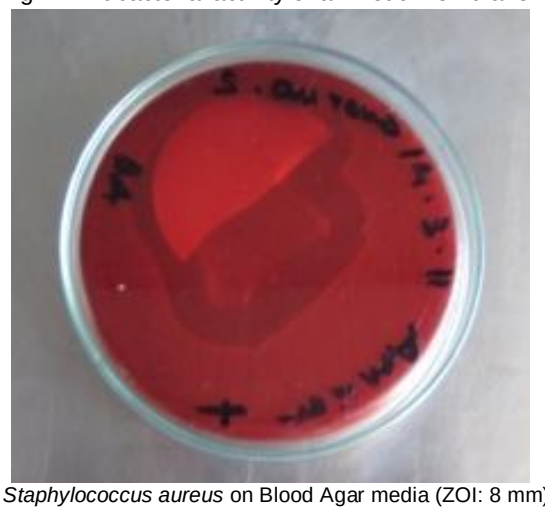
Fungal pathogens	Amniotic membrane				Chorionic membrane			
	$5X10^5$	$5X10^6$	$5X10^7$	$5X10^8$	$5X10^5$	$5X10^6$	$5X10^7$	$5X10^8$
Aspergillus niger	+++	+++	+++	+++	+++	+++	++	++
Aspergillus flavus	+++	+++	++	+	++	++	++	+
Aspergillus nidulans	+++	+++	+++	+	+++	+++	+++	+
Aspergillus fumigatus	+++	+++	+++	++	++	++	++	+

Antimicrobial activity: In this method, 0.5 McFarland standard is prepared by mixing 0.05 mL of 1.175% barium chloride dihydrate with 9.95 mL of 1% sulfuric acid. The 24 h old broth culture of bacterial and 48 h old culture of fungal strains were separately dissolved in 0.5 McFarland suspensions and serially diluted up to $1.5X10^8$ and the dilutions such as $5X10^5$, $5X10^6$, $5X10^7$ and $5X10^8$ were uniformly spread over the nutrient or blood agar medium plate by using sterile swabs. Then a small piece of the amniotic membrane and chorionic membrane were separately placed in center of each plate and incubated for 24 h at 37°C. After the incubation period, the zone of inhibition (ZOI) around the membranes was measured (Soltan *et al*., 2013).

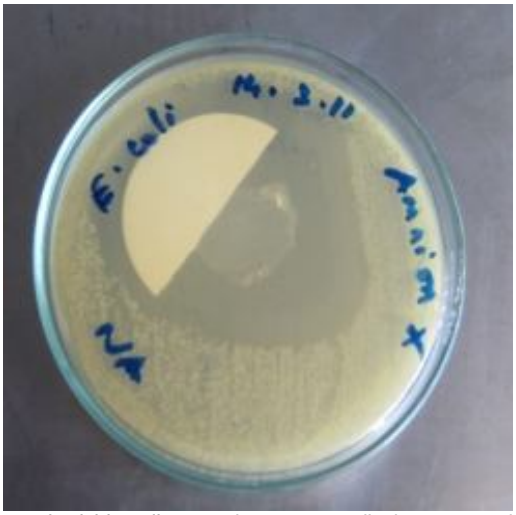
Results and discussion

In general, there are some natural antimicrobials produced in amniotic fluid during pregnancy and the epithelial layer consists of human beta-defensins 1-3 (HBD), elafin and secretory leukocyte protease inhibitor (SLPI), among that HBD-2 is a strong antibiotic and is expressed in response to IL-1 in amniotic epithelial cells (Stock *et al*., 2007; King *et al*., 2007). The antimicrobial properties of amniotic fluid may be due to the presence of lysozymes, 7S immunoglobulin and IgA (Thadepalli *et al*., 1978; Cunha *et al*., 1984). The maximum zone of inhibitory activity was found in *Staphylococcus aureus, Escherichia coli* and *Shigella* sp. by both fetal membranes (Table 1; Fig. 1a, b; 2a, b). In each of the four studied dilutions, the similar diameter of inhibition zone was observed in $1.5X10^5$ and $1.5X10^6$ dilutions. While assessing the zone of inhibition against fungal pathogens the maximum zone was found in *Aspergillus niger* and *A. nidulans* by both fetal membranes (Table 2). In each of four studied dilutions, the similar diameter of inhibition zone was observed in $1.5X10^5$ and $1.5X10^6$ dilutions.

Fig. 1. Antibacterial activity of amniotic membrane.

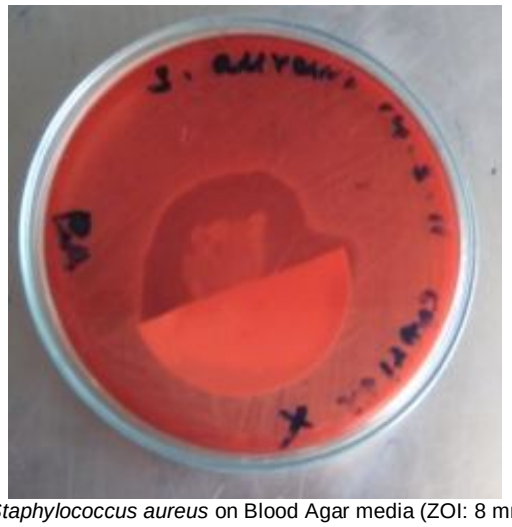


a. *Staphylococcus aureus* on Blood Agar media (ZOI: 8 mm).

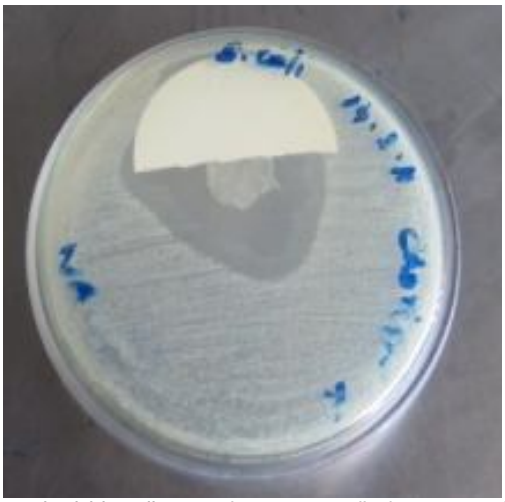


b. *Escherichia coli* on Nutrient Agar media (ZOI: 12 mm).

Fig. 2. Antibacterial activity of chorionic membrane.



a. *Staphylococcus aureus* on Blood Agar media (ZOI: 8 mm).



b. *Escherichia coli* on Nutrient Agar media (ZOI: 12 mm).

Similar results were observed by Kjaergaard *et al*. (2001) who reported antibacterial effect of amniotic and chorionic membranes on strains of *Streptococcus* group A and B*, Staphylococcus aureus, S. saprophyticus* and *Enterococcus faecalis.* Maximum inhibition zone diameter was found in *Streptococcus* Group A*, Staphylococcus aureus* and *S. saprophyticus*. Talmi *et al.* (1991) also proved the similar results in his study when assessing the antimicrobial activity against *Streptococcus* Group B microbial suspension at different concentrations. In this study, the microbial suspension was more dilute than their suspension and also it is well established that the reduction of dilution did not affect the inhibitory results and it confirmed that the antimicrobial effect of human amniotic and chorionic membrane is stable against various dilutions of the microbial suspensions. While assessing the zone of inhibition against fungal pathogens, the maximum zone was found in *Aspergillus niger* and *A. nidulans* by both membranes (Table 2).

Conclusion

The present study clearly confirmed the antimicrobial effect of both amniotic and chorionic membranes against several bacterial and fungal pathogens. Among the two membranes, the maximum activity was recorded by amniotic membrane. Consequently the study also suggested that both membranes can be used as a biological material in tissue engineering and other medical fields in near future.

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