



Predictive Study of Serum Matrix Metalloproteinase-8 in Combination with Human Chorionic Gonadotropin in Preterm Amniotic Cavity Infection

Yashu Lv*, Yueyue Gao, Jinyi Wang, Yunchun Liu and Guiqing Jiao

Department of Obstetrics and Gynecology, The First Affiliated Hospital of Hebei North University, Zhangjiakou, Hebei075000, China

ABSTRACT

The objective of this study was to investigate the predictive value of serum matrix metalloproteinase-8 (MMP-8) combined with human chorionic gonadotropin (hCG) for preterm amniotic cavity infection. The study included ninety patients with preterm labor admitted from June 2021 to October 2022 in the First Affiliated Hospital of Hebei North University, including the premature rupture of membranes group and the intact fetal membranes group. The same number of pregnant women with the same gestational age as in the experimental group were randomly selected as the study subjects in the outpatient clinic. Maternal sera from both groups β -hCG, MMP-8 levels were expressed and compared with those in the group with intra amniotic infection and no intra amniotic infection β - Higher levels of hCG, MMP-8 expression ($P < 0.05$; Placentas from both groups β -hCG, MMP-8 levels were expressed and compared with those in the group with intra amniotic infection and no intra amniotic infection β - Higher levels of hCG, MMP-8 expression were factors associated with the development of preterm intra amniotic infection ($P < 0.05$). It was concluded that in the serum of patients with preterm amniotic cavity infection, MMP-8 β - The values of hCG showed obvious expression by detecting MMP-8 β -hCG expression, which helps to predict the presence of intraamniotic infection, and especially for the prediction of subclinical intraamniotic infection is of high clinical significance. MMP-8 and β - The combined hCG test can provide clinical data support for clinicians to fully judge the prognosis of mother and child and to administer clinical interventions as early as possible, with high diagnostic efficacy.

Article Information

Received 14 February 2023

Revised 28 February 2023

Accepted 13 March 2023

Available online 20 July 2023
(early access)

Published 10 November 2024

Authors' Contribution

YL, YG and JW conducted the experiments in this study. YL and GJ contributed to the design and interpretation of the current study and wrote the article. All authors read, revised, and approved the final manuscript.

Key words

β -human chorionic gonadotropin, Matrix metalloproteinase-8, Intraamniotic infection, Preterm delivery, Premature rupture of membranes

INTRODUCTION

Premature preterm rupture of membrane (PPROM) occurs in 3% of pregnancies and is the cause of about 25-30% of all premature births and is one of the important causes of perinatal morbidity (Mercer, 2007; Weissmann-Brenner *et al.*, 2009; Blott and Greenough, 1988). One of the most important reasons for the importance of PPRM is its association with the short interval between the time of rupture of the membranes and childbirth, and this issue is of great importance due to the birth of premature babies in PPRM (Mercer, 2007). During the time interval between

water sac rupture and childbirth, the possibility of pathogenic microorganisms climbing from vagina to amnion cavity increases and it is believed to play a role in the increase of intrauterine infection (Pasquier *et al.*, 2009; Keyon *et al.*, 2001; Gopalani *et al.*, 2004; Yoon *et al.*, 1999; Lajos *et al.*, 2008).

In some sources, PPRM has been introduced as a pathological process that mostly occurs following inflammation and infection of membranes. In histological studies of membranes after premature rupture of membranes, specific bacterial contamination along the choriodecidual surfaces with brief amnion involvement is indicated. Also, in women, there is a high PPRM incidence of positive culture of amniotic fluid (25-30%) in amniocentesis samples, even when there is no clinical suspicion of chorioamnionitis (Mercer, 2007; Cunningham *et al.*, 2005). Therefore, one of the major risks in PPRM patients is the occurrence of uterine infection, which leads to complications such as chorioamnionitis, postpartum metritis, and perinatal complications such as neonatal sepsis (Mercer, 2007; Yoon *et al.*, 1999).

Premature rupture of the membranes in preterm labour

* Corresponding author: hxgszwh@163.com
0030-9923/2024/0000-3059 \$ 9.00/0



Copyright 2024 by the authors. Licensee Zoological Society of Pakistan.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

leaves the amniotic cavity in a state of communication with the outside world and allows amniotic fluid to escape, which in turn leads to amniotic cavity infection in the mother (Zhang *et al.*, 2021). Most amniotic cavity infections are due to the ability of maternal vaginal bacteria to travel retrograde to the amniotic cavity through the amniotic rupture after the fetal membranes have ruptured (Zuo *et al.*, 2021). Most scholars believe that amniotic cavity infection, is one of the main factors leading to a range of complications and sequelae in pregnant women and newborns (Fan *et al.*, 2021).

The early stages of amniotic cavity infection in pregnant women with preterm labour are usually not clearly specific. Therefore, it is difficult to detect amniotic cavity infection earlier and then to intervene in time for the subsequent treatment of the pregnant woman (Fang *et al.*, 2021). Cellular signaling plays an important role in the specific pathogenesis of preterm amniotic cavity infection (Yang and Lei, 2020). In this paper, we chose to analyse β -human chorionic gonadotropin (β -hCG) combined with serum matrix metalloproteinase-8 (MMP-8) expression in mothers with preterm amniotic cavity infection, in order to provide clinical data to support clinicians in predicting the prognosis of mothers and infants and to reduce the occurrence of serious complications through early clinical intervention.

MATERIALS AND METHODS

Study design

Ninety cases of preterm delivery patients from June 2021 to October 2022 in our hospital were collected for the study. The 90 cases were divided into no amniotic infection group and amniotic infection group by age detection of amniotic infection. There were 46 patients in the uninfected group, age range 22-40 years, with a mean of 29.98 ± 2.28 years, gestational weeks 19-32 weeks, with a mean of 25.29 ± 1.44 weeks, and a history of preterm delivery in 6 patients. There were 44 patients in the infected group, age range 24-42 years, with a mean of 29.95 ± 2.33 years, gestational weeks 20-31 weeks, with a mean of 25.34 ± 1.42 weeks, and a history of preterm delivery in 2 patients. There were no significant differences between the two groups in terms of age, number of weeks of gestation and testing methods, which was comparable. All subjects signed an informed consent form and the study was approved by the ethics committee of our hospital.

Patients in both groups were eligible to join the study if they met the following inclusion criteria: Meets diagnostic criteria related to preterm birth, all singleton pregnancies below 20 weeks gestation, normal mental status, normal comprehension and cognitive ability. Pregnant women

with primary hypertension, diabetes mellitus, heart disease and severe liver and kidney abnormalities were excluded from both groups.

At the time of admission and 1 day after delivery, 5 ml of fasting venous blood was collected in the early morning h. The venous blood was centrifuged in a serum centrifuge at 3000 r/min for 10 min and the serum was removed.

The fully automated biochemical analyser BECKMAN AU 5800 was used for the assay. The β -hCG and MMP-8 assay was performed by an enzyme-linked immunoassay. The reagents were used from Mike's Biologicals Co., Ltd. and the instrument was HITACH008, a fully automatic biochemical analysis instrument from Japan. The antigen was diluted by adding 50 ml of carbonate buffer to the polystyrene wells and stored under cover in a refrigerator at a constant temperature of 4°C for 24 h. The wells were then washed 3 times the next day and patted dry. The specimens were diluted with 0.02 mol/L Tris-HCl pH 7.4 buffer and placed in each well. Positive and negative control specimens were placed in the wells and stored at 42°C for 60 min. After removal of the liquid, the wells were washed 3 times and dried. 0.1 ml of β -hCG and MMP-8 antibodies were placed in each well, held for 60 min, and then the liquid was removed. The wells were washed 3 times and then blotted dry. All wells were placed in the substrate solution (0.1 mol/L Na_2HPO_4 , 0.05 mol/L citric acid) and 0.1 mL of o-phenylenediamine was added after homogenization. After shading the wells for 20 min, 2 mol/L H_2SO_4 0.05 ml was placed in the wells and the reaction was terminated. The levels of β -hCG and MMP-8 were analyzed by measuring A450 values with an enzyme marker.

Statistical analyses

SPSS 21.0 software was used for processing. LSD-t test was performed for comparison between groups. The measurement data were described by applying ($\bar{x} \pm s$) and the count data were expressed as percent. Correlation analysis was performed by Spearman, while $P < 0.05$ indicated statistical significance.

RESULTS

The expression of maternal serum β -hCG and MMP-8 levels in both groups are shown in Table I. The expression of β -hCG and MMP-8 levels are higher in the amniotic cavity infection group compared to the group without amniotic cavity infection, with statistical differences ($P < 0.05$). The expressions of placental β -hCG and MMP-8 levels in the two groups are shown in Table I. Compared with the group without amniotic cavity infection, the expression of β -hCG and MMP-8 levels are higher in the amniotic cavity

infection group, with statistical differences ($P < 0.05$).

Table I. Expression analysis of β -hCG and MMP-8 levels in the two groups ($\bar{x} \pm s$).

Group	Serum		Placental	
	β -hCG ($\mu\text{mol/L}$)	MMP-8 (pg/mL)	β -hCG ($\mu\text{mol/L}$)	MMP-8 (pg/mL)
Uninfected ($n = 46$)	18.04 $\pm$ 1.21	19.24 $\pm$ 4.23	1.04 $\pm$ 0.11	1.14 $\pm$ 0.13
Infected ($n = 44$)	29.25 $\pm$ 2.07	31.27 $\pm$ 3.51	4.25 $\pm$ 0.37	3.27 $\pm$ 0.41
t	31.531	14.647	56.310	33.525
P	0.001	0.001	0.001	0.001

As shown in Table II, the diagnostic efficacy of β -hCG and MMP-8 in the diagnosis of intra-amniotic infection in preterm labour was found to be higher in terms of specificity and sensitivity, as well as the Jorden index, while the combination of the two was significantly higher than the diagnostic efficacy of the single index.

Table II. Efficacy of β -hCG and MMP-8 levels expression in the diagnosis of intra-amniotic infection in preterm labour.

Factors	Optimal cut-off	AUC	Sensitivity (%)	Specificity (%)	Jorden index (%)
β -hCG	6.852	0.751	0.855	0.842	0.829
MMP-8	4.326	0.594	0.842	0.857	0.846
Combined test	-	0.854	0.925	0.914	0.843

Table III. Multifactorial logistic regression analysis of intra-amniotic infection in preterm labour.

Risk factors	Beta	SE	Wald	P value	OR value	95%CI
β -hCG	1.626	0.856	5.225	0.001	3.326	1.542-6.621
MMP-8	1.431	0.18	4.245	0.001	2.586	1.151-5.338

As shown in Table III, the occurrence of intra-amniotic infection in preterm labour was included as the dependent variable and β -hCG and MMP-8 were included as independent variables in a multi-factor logistic regression model analysis. Both β -hCG and MMP-8 were found to be factors in the occurrence of intra-amniotic infection in preterm labour ($p < 0.05$).

DISCUSSION

The mechanism of preterm delivery is a hot topic of

research in perinatal medicine, but the etiology leading to preterm delivery has not been comprehensively elucidated (Wang *et al.*, 2020). Relevant studies have shown that preterm labour may be one of the clinical manifestations of most pregnancy complications. The association with infection, a recognized cause of preterm labour, becomes more profound the earlier the gestational week in which preterm labour occurs (Li *et al.*, 2020). Infection may lead to the activation of a variety of pro-inflammatory cytokines, which in turn may result in a disturbance of the immune balance at the maternal-fetal interface, thus causing preterm birth (Chen *et al.*, 2018). With advances in medical technology and the use of various testing techniques and immunology disciplines in obstetrics and gynaecology, the techniques for diagnosing premature rupture of the membranes and intra-amniotic infection have matured (Ma *et al.*, 2020). Among these, maternal blood CRP is widely used in clinical obstetric investigations, but reliance on CRP as a predictor is inadequate. Foreign studies have found that intra-amniotic infection is not significantly associated with CRP level expression, so the search for cytokines with better diagnostic value is significant (Zhou *et al.*, 2020).

It has been demonstrated that inflammatory mediators, such as CRP/PCT, can be elevated in the serum of pregnant women with amniotic cavity infection. MMP-8 is a cellular matrix-degrading enzyme that is stimulated by inflammatory factors that can contribute to the release of MMP-8. High expression of inflammatory factors can impair fetal defences and promote prostaglandin secretion, which in turn can contribute to fetal injury by the immune system and induce preterm labour (Holmström *et al.*, 2019; Gulbinienė *et al.*, 2022). β -hCG is a glycoprotein hormone secreted by placental trophoblast cells during pregnancy. Hormone levels rise significantly when a pregnant woman has damaged fetal membranes or an infection in the amniotic cavity (Liao *et al.*, 2020). In the existing studies, only a single study was conducted for the above infection indicators, which lacked better accuracy.

MMP-8, also known as neutrophil collagenase, is synthesised by bone marrow neutrophils during the maturation phase. It has been found that during normal pregnancy, amniotic fluid does not contain MMP-8, but it is expressed in cervical tissue, where it has a facilitative effect on cervical maturation (Li *et al.*, 2019). The absence of leukocytes, that is, neutrophils, in the amniotic fluid of normal pregnant women means that there is no expression of MMP-8 in the amniotic fluid of normal pregnant women, but the presence of infection in the amniotic cavity, where neutrophils proliferate and secrete large amounts of MMP-8, suggests that MMP-8 could be used as a specific indicator of the level of inflammation in the amniotic cavity (Zhou,

2019). The placenta not only serves as a site of material exchange and nutrient metabolism between the mother and fetus, but also secretes hormones, acts as a pathogen barrier and is an innate immune organ during pregnancy (Choi *et al.*, 2023; Myntti *et al.*, 2017). Studies have shown that intrauterine infections are closely associated with a variety of pregnancy complications, of which placental infections will affect fetal outcome (Pittayapruerk *et al.*, 2016). MMP-8, the most important collagenase of type I collagen, is prone to secrete inflammatory factors in large amounts when the uterine cavity is attacked by inflammatory mediators, and high levels of inflammation can activate the neutrophil-secreting MMP-8 pathway, which manifests itself as an overexpression of MMP-8 in the amniotic fluid (Zhang *et al.*, 2020). When MMP-8 breaks down type I collagen in large quantities, the elasticity, tone and strength of the amnion are severely affected, leading to structural weakening of the fetal membranes and premature rupture of the membranes. Premature rupture of the fetal membranes is associated with intra-amniotic infection, creating a vicious cycle that leads to poor pregnancy outcomes (Balciuniene *et al.*, 2021; Chaemsaitong *et al.*, 2018).

CONCLUSION

The values of MMP-8 and β -hCG were significantly expressed in the serum of patients with premature amniotic cavity infection. Detection of MMP-8 and β -hCG expression can help to predict the presence of amniotic cavity infection, especially for subclinical amniotic cavity infection. The combination of MMP-8 and β -hCG can provide clinical data to support the clinician's overall assessment of maternal and infant prognosis and early clinical intervention, with high diagnostic efficacy.

ACKNOWLEDGEMENT

Authors would like to thank Professor Zhang for his expert advice and encouragement. He has helped me complete many difficult tasks, and also want to thank Dr. Ma for his great help in the laboratory.

Funding

Self-funded project of Zhangjiakou Science and Technology Bureau in 2021 (Fund N.O: 2121072D).

IRB approval

This study was approved by the First Affiliated Hospital of Hebei North University, Zhangjiakou, Hebei 075000, China.

Ethical approval

The study was carried out in compliance with guidelines issued by ethical review board committee of The First Affiliated Hospital of Hebei North University, Zhangjiakou, Hebei 075000, China. The official letter would be available on fair request to corresponding author.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

- Balciuniene, G., Gulbinienė, V., Dumalakiene, I., Viliene, R., Bartkeviciene, D., Pilypiene, I., Drasutiene, G.S. and Ramasauskaite, D., 2021. Prognostic markers for chorioamnionitis: IL-6, TNF- α , and MMP-8 in vaginally obtained amniotic fluid. *J. clin. Med.*, **10**: 1136. <https://doi.org/10.3390/jcm10051136>
- Blott, M. and Greenough, A., 1988. Neonatal outcome after prolonged rupture of the membranes starting in the second trimester. *Arch. Dis. Child.*, **63**: 1146-1150. https://doi.org/10.1136/adc.63.10_Spec_No.1146
- Chaemsaitong, P., Romero, R., Docheva, N., Chaiyasit, N., Bhatti, G., Pacora, P., Hassan, S.S., Yeo, L. and Erez, O., 2018. Comparison of rapid MMP-8 and interleukin-6 point-of-care tests to identify intra-amniotic inflammation/infection and impending preterm delivery in patients with preterm labor and intact membranes. *J. Matern. Fetal. Neonatal. Med.*, **31**: 228-244. <https://doi.org/10.1080/14767058.2017.1281904>
- Chen, G., Li, G. and Zou, Y.R., 2018. Predictive significance of serum ICAM-1 and IL-6 assay combined with immunofluorescent staining for PPRM combined with amniotic cavity infection. *China Mat. Child Hlth. Res.*, **29**: 1018-1022.
- Chen, S.E., Chen, X.Y. and Chen, C., 2021. Impact of premature rupture of membranes on the early prognosis of ultra-preterm infants. *Chin. J. Contemp. Ped.*, **23**: 25-30.
- Choi, S.R., Kim, T., Kim, Y., Jung, S. and Choi, S.J., 2021. The relationship between matrix metalloproteinase-8 in after birth oral fluid and acute histologic chorioamnionitis in preterm delivery. *Reprod. Sci.*, **28**: 2023-2028. <https://doi.org/10.1007/s43032-020-00448-4>
- Cunningham, F.G., Leveno, K.J., Bloom, S.L., Hauth, J.C., Gilstrap, L. and Wenstrom, K.D., 2005. *Williams obstetrics*. 22th ed. McGraw Hill, New York. pp. 232-247.

- Fan, Y.Y., Wang, N. and Xu, W., 2021. Preventive effect of fresh amniotic membrane on uterine adhesions and improvement of endometrial tolerance in rabbits. *J. Jilin Univ.*, **47**: 990-998.
- Fang, X., Lu, Y.Y. and Zhao, S., 2021. Advances in the study of chorioamnionitis and adverse outcomes in preterm infants. *Elect. J. Dev. Med.*, **9**: 151-155.
- Gopalani, S., Krohn, M., Meyn, L., Hitti, J. and Crombleholme, W.R., 2004. Contemporary management of preterm premature rupture of membranes: Determinants of latency and neonatal outcome. *Am. J. Perinatal*, **21**: 183-190. <https://doi.org/10.1055/s-2004-828609>
- Gulbinienė, V., Balciuniene, G., Dumalakiene, I., Viliene, R., Pilypiene, I. and Ramasauskaite, D., 2023. The significance of TNF- α and MMP-8 concentrations in non-invasively obtained amniotic fluid predicting fetal inflammatory response syndrome. *Int. J. Gynecol. Obstet.*, **160**: 476-482. <https://doi.org/10.1002/ijgo.14478>
- Holmström, E., Myntti, T., Sorsa, T., Kruit, H., Juhila, J., Paavonen, J., Rahkonen, L. and Stefanovic, V., 2019. Cervical and amniotic fluid matrix metalloproteinase-8 and interleukin-6 concentrations in preterm pregnancies with or without preterm premature rupture of membranes. *Fetal. Diagn. Ther.*, **46**: 103-110. <https://doi.org/10.1159/000493207>
- Kenyon, S.L., Taylor, D.J. and Tarnow-Mordi, W., 2001. Broad-spectrum antibiotics for preterm, prelabour rupture of fetal membranes: The ORACLE I randomized trial. *Lancet*, **357**: 979-988. [https://doi.org/10.1016/S0140-6736\(00\)04233-1](https://doi.org/10.1016/S0140-6736(00)04233-1)
- Lajos, G.J., Passini-Junior, R., Nomura, M.L., Amaral, E., Pereira, B.G. and Milanez, H., 2008. Cervical bacterial colonization in women with preterm labor or premature rupture of membranes. *Rev. Bras. Ginecol. Obstet.*, **30**: 393-399. <https://doi.org/10.1590/S0100-72032008000800004>
- Li, D.H., Peng, X.L. and Ma, Q.X., 2019. Predictive value of serum human chorionic gonadotropin combined with ultrasensitive C-reactive protein levels for prenatal amniotic cavity infection. *China Mater. Child. Hlth.*, **34**: 512-514.
- Li, J.M., Lin, J. and Peng, Y.N., 2020. Report of a case of severe uterine adhesions treated with decellularized amniotic membrane carrier compounded with autologous endometrial stem cells. *J. Rep. Med.*, **29**: 541-544.
- Liao, H.B., Wei, L. and Cheng, X.H., 2020. Serum MMP-2, IL-8, and β -hCG in the diagnosis of combined amniotic cavity infection in patients with prematurely ruptured fetal membranes. *Chin. J. Hosp. Infect.*, **30**: 1393-1397.
- Ma, X.L., Ma, Z.F. and Zhao, W.B., 2020. Changes in CRP, IL-6, sPLA2, HBD-2 and clinical significance in patients with premature rupture of the amniotic cavity in preterm fetus. *Chin. J. Hosp. Infect.*, **30**: 1432-1435.
- Mercer, B.M., 2007. Premature rupture of the membrane. In: *Complicated pregnancy* (eds. F. Petraglia and G.F. Strauss). 4th ed. Information Health Care, London. pp. 713-727. <https://doi.org/10.1016/B978-0-443-06930-7.50029-3>
- Myntti, T., Rahkonen, L., Nupponen, I., Pätäri-Sampo, A., Tikkanen, M., Sorsa, T., Juhila, J., Andersson, S., Paavonen, J., Stefanovic, V., 2017. Amniotic fluid infection in preterm pregnancies with intact membranes. *Dis. Markers*, **2017**: 8167276. <https://doi.org/10.1155/2017/8167276>
- Pasquier, J.C., Picaud, J.C., Rabilloud, M., Claris, O., Ecochard, R., Moret, S. and Mellier, G., 2009. Neonatal outcomes after elective delivery management of preterm premature rupture of the membranes before 34 weeks gestation (DOMINOS study). *Eur. J. Obstet. Gynecol. Rep. Biol.*, **143**: 18-23. <https://doi.org/10.1016/j.ejogrb.2008.10.017>
- Pittayapruk, P., Meephansan, J., Prapapan, O., Komine, M. and Ohtsuki, M., 2016. Role of matrix metalloproteinases in photoaging and photocarcinogenesis. *Int. J. mol. Sci.*, **17**: 868. <https://doi.org/10.3390/ijms17060868>
- Wang, L.Y., Dong, K. and Xiong, W.D., 2020. Diagnostic value of combined detection of serum amyloid A and calcitoninogen in amniotic cavity infection in pregnant women with premature rupture of membranes. *Chin. J. Hlth. Test.*, **30**: 257-259.
- Weissmann-Brenner, A., O'Reilly-Green, C., Ferber, A. and Divon, M.Y., 2009. Values of amniotic fluid index in cases of preterm premature rupture of membranes. *J. Perinat. Med.*, **37**: 232-235. <https://doi.org/10.1515/JPM.2009.078>
- Yang, Y. and Lei, X.B., 2020. Effect of serum sPLA2 and HBD-2 on preterm premature rupture of fetal membranes and amniotic cavity infection. *Chin. J. Hosp. Infect.*, **30**: 1398-1401.
- Yoon, B.H., Kim, Y., Romero, R., Kim, J.C., Park, K.H. and Kim, M.H., 1999. Association of oligohydramnios in women with preterm premature Rupture of membranes with an inflammatory response in fetal, amniotic, and maternal compartments. *Am. J. Obstet. Gynecol.*, **181**: 784-788. [https://doi.org/10.1016/S0002-9378\(99\)70301-7](https://doi.org/10.1016/S0002-9378(99)70301-7)

- Zhang, W.L., Yang, L. and Liu, N.N., 2020. Study on the relationship between MMP-8, MPO and HCMV infection and AS plaque type in ICVD patients. *Chin. J. exp. clin. Virol.*, **34**: 636-639.
- Zhou, L., 2019. Progress of IL-1 β , MMP-8 and fetal thymus in predicting premature rupture of membranes combined with chorioamnionitis. *Int. J. Obstet. Gynecol.*, **46**: 44-47.
- Zhou, Q.M., Zheng, L.M. and Xiao, M.F., 2020. Clinical value of serum LBP and β -hCG and secretory GBS in predicting premature rupture of membranes combined with amniotic cavity infection. *Chin. J. Hosp. Infect.*, **30**: 2816-2820.
- Zuo, J.Y., Wang, X.L. and Chai, Y.Y., 2021. Predictive value of combined vaginal irrigation fluid cytokine and gestational age test for amniotic cavity infection. *J. Hunan Nor. Univ.*, **18**: 268-271.