BACTERIAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF AEROBIC VAGINAL PATHOGENS IN GYNAE PATIENTS VISITING TO KHYBER TEACHING HOSPITAL PESHAWAR

Muhammad Asghar[1], Naheed[2], Aakash Ahmad Khattak[3], Sadiq Azam[4], Noor Rehman[5], Ibrar Khan[6], Gul-e-Sehra[7], Anila Farid[8]

ABSTRACT:

OBJECTIVES:

The aim of this study was to determine the prevalent aerobic vaginal bacteria and their antibiogram to commonly prescribed antibiotics for the treatment of aerobic vaginitis (AV).

METHODOLOGY:

A total of 200 high vaginal swabs (HVS) samples were collected from different AV suspected patients visiting Khyber Teaching Hospital (KTH) and processed for identification of bacterial isolates followed by antibiotic susceptibility patterns as per CLSI protocols.

RESULTS:

Out of 200 clinical samples, 70 (35%) HVS isolates yielded bacterial growth. Of the isolates, E.coli was the common pathogen 36 (51.4%) followed by S.aureus 20 (28.5%), Enterobacter spp 08 (11.4%), Pseudomonas spp 04 (5.7%) and Citrobacter spp 02 (2.8%). The highest prevalence was observed in the age group of 21-35 years (31.4%) followed by age groups 16-20 years (25.7%) and 26-30 years. S.aureus isolates (n=20) were resistant to ciprofloxacin (90%), cephradine (70%), erythromycin (70%), gentamicin (50%) and cefotaxime (40%) while 1 (5%) of each isolate was resistant to methicillin and vancomycin. Majority of the gram-negative isolates (n=50) were resistant to cotrimoxazole, cephalosporins, quinolones, aminoglycosides and susceptible to carbapenems, tigecycline, sulbactam and tazobactam.

CONCLUSION:

Aerobic vaginitis should be treated very selectively in order not to kill the beneficial bacteria. Before treating AV, the causative agents should be accurately identified and tested for drug susceptibility patterns and empirical antibiotic therapy should be avoided.

KEYWORDS: Aerobic Vaginal Pathogens, Aerobic Vaginitis, Antibiogram

Correspondence:
[5]Noor Rehman, Microbiologist, Department of Pathology, Khyber Teaching Hospital, Peshawar,
Contact: +92-333-9535336
Email: noorbangash61@gmail.com
[1]Assistant Professor, Department of Pathology, Khyber Medical College/Khyber Teaching Hospital, Peshawar.
[2]Assistant Professor, Department of Dermatology, Hayatabad Medical Complex, Peshawar
[3]Biotechnologist, Department of Pathology, Khyber Medical College/Khyber Teaching Hospital, Peshawar
[4]Assistant Professor, Centre of Biotechnology and Microbiology, University of Peshawar.
[6]Associate Professor, Centre of Biotechnology and Microbiology, University of Peshawar
[7]Lecturer, Centre of Biotechnology and Microbiology, University of Peshawar
[8]Demonstrator, Abbottabad International Medical College, Abbottabad

INTRODUCTION:

Aerobic Vaginitis (AV) is characterized by disturbance of normal flora of female vagina followed by replacement of opportunistic microorganisms found in intestine like staphylococcus spp, E.coli and group B streptococci, parabasal cells and vaginal leukocytes. AV was initially reported by Donders et al in 2002[1]. It is clinically diagnosed by the occurrence of immature epithelial cells, a disturbed lactobacilli population, growth of aerobic micro flora and inflammation that provoke a localized vaginal inflammatory immune response[2]. The AV patients have clinical symptoms; vaginal discharge, pain during and after sexual activity, prickling and burning sensation, and a strong inflammatory response resulting miscarriage, premature labor and delivery[3]. Vaginal bacterial microorganisms either contain lacto-bacillary morph types in case of normal flora or can be abnormal. Abnormal vaginal flora is dominated by an overgrowth of microorganisms that do not require oxygen (BV) or by oxygen-consuming organisms, such as E.coli, group B streptococci and enterococci etc. (AV), or can be a combination of both AV and BV[4]. In a study, consisting of 631 patients attending routine prenatal assistance from a vaginitis clinic, 7.9% had mild to extreme AV symptoms, while 6% had full-blown BV[1]. Another study conducted in Pakistan reported the high prevalence of AV (78%) as compared to other epidemiological studies. They observed the prevalence of different bacterial agents: staphylococcus aureus (46%) among which 35% were (MRSA),

escherichia coli (25%), klebsiella pneumoniae (16%), enterococcus faecalis (6%), pseudomonas species (5%) and proteus spp (2%)[5]. The clinicians suggested empirical treatment for 5-7 days but the patients were rarely recovered. Therefore it is not clear if severe and recurrent therapies will be effective or not. Further investigation should be done for establishing optimum procedures for therapeutic purposes. It was reported that local kanamycin against non-pregnant females suffering from AV resulted in successful treatment therapy for the enterobacteriaceae family after culture and antibiogram[6]. Another promising treatment with the use of local, non-absorbable antibiotics is preferred due to their ability to stimulate the desired anti-inflammatory environment[7]. Hoyme and Saling conducted a study in a non-randomized manner using a group of people with normal vaginal flora as control[8]. A screening system was installed for self-measurement of vaginal pH and in case of abnormal vaginal flora treatment was also done. The study revealed that by treating abnormal vaginal flora, a much larger decrease in premature birth was observed[9]. Bacterial vaginosis is an issue of concern for clinicians to overcome the bacterial vaginitis and their associated risk factors among pregnant women. The current study is therefore designed to identify the causative agents of aerobic vaginitis and determine their resistance pattern.

METHODOLOGY:

A cross sectional study was conducted at the Centre of Biotechnology and Microbiology, University of Peshawar, and Pathology Department, KTH, Peshawar. All the 200 HVS samples were recovered from female gynae patients having complaints of an itching or burning sensation, vaginal inflammation, dyspareunia and yellowish discharge (16-43 years). Those patients excluded from this study were taking any antibiotic or had other known infections (parasitic infestations, fungal infection). A total of 200 HVS samples were obtained using sterile cotton swabs from AV patients from gynae wards and labor rooms and transported to the Microbiology section of the Pathology Department KTH for further processing. The collected HVS samples were inoculated on Blood, MacConkey, and Mannitol Salt Agar (MSA) media (Oxoid, UK) plates and

incubated overnight at 37°C. Preliminary, bacteria were identified based on physical appearances; lactose fermenters and non lactose fermenters on MacConkey agar, blood agar (hemolysis), MSA medium (changes in physical appearance) and biochemical tests (catalase, coagulase, oxidase). Gram-positive Cocci (GPC) with positive coagulase, catalase and deoxyribonuclease (DNase) activity were identified as S.aureus while Cefoxitin disc was used to identify MRSA, as it is resistant to this antibiotic[10]. Gram-negative bacilli (GNR) were identified by biochemical tests using Analytical Profile Index (API) 10S strips (Biomerieux, France)[11]. Antibiogram was done for all the isolates against selected antibiotic discs by disc diffusion technique as per CLSI-2019 guidelines. The bacterial suspension was adjusted to 0.5 McF and spread on MHA. The results were interpreted after overnight incubation at 37°C by measuring the inhibition zone diameter according to CLSI interpretive criteria[12]. The antibiotics; ciprofloxacin (CIP), gentamicin (CN), tygacil (TGC), linezolid (LZN), vancomycin (VA), fusidic acid (FA), erythromycin (E), cefoxitin (FOX), amikacin (AK), cefotaxime (CTX), cefaclor (CEF) and cephradine (CE) used on Gram-Positive Bacteria/Cocci (GPC) while those used on Gram-Negative Bacteria/Rods (GNR) were; co-amoxiclav (AMC), CIP, CN, meronem (MEM), imipenem (IPM), sulzone (SCF), tazocin (TZP), cefotaxime (CTX), chloramphenicol (C), AK and aztreonam (ATM), TGC and cotrimoxazole (SXT).

RESULTS:

A total of 200 HVS samples were recovered from various suspected patients having aerobic vaginitis infection.

Table 1: Prevalence of AV Isolates and Distribution of Different Age Groups among Positive Isolates

Parameters	AV Positive Isolates	Percentage (%)
Total Positive Isolates	70	35
Prevalence of AV Isolates		
E.coli	36	51.4
S.aureus	20	28.5
Enterobacter spp	08	11.4
Pseudomonas spp	04	5.7
Citrobacter spp	02	2.8
Age Group		
10-15 years	02	2.8
16-20 years	18	25.7
21-25 years	22	31.4
26-30 years	16	22.8
>30 years	12	17.1

Table 2: Antibiogram of MDR Isolates Recovered from Aerobic Vaginitis Patients

| Antibiotics	E.coli (n=36)		Enterobacter spp (n=8)		Pseudomonas spp (n=4)		Citrobacter spp (n=2)		S.aureus (n=20)	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
MEM	34 (94.4)	02 (5.5)	06 (75)	02 (25)	04 (100)	00 (00)	02 (100)	00 (00)	-	-
SCF	32 (88.8)	04 (11.1)	06 (75)	02 (25)	02 (50)	02 (50)	02 (100)	00 (00)	-	-
TZP	32 (88.8)	04 (11.1)	06 (75)	02 (25)	02 (50)	02 (50)	02 (100)	00 (00)	-	-
ATM	18 (50)	18 (50)	04 (50)	04 (50)	00 (00)	04 (100)	00 (00)	02 (100)	-	-
SXT	02 (5.5)	34 (94.4)	02 (25)	06 (75)	00 (00)	04 (100)	01 (50)	01 (50)	-	-
TGC	30 (83.3)	06 (16.6)	06 (75)	02 (25)	04 (100)	00 (00)	02 (100)	00 (00)	20 (100)	00 (00)
IPM	34 (94.4)	02 (5.5)	06 (75)	02 (25)	04 (100)	00 (00)	02 (100)	00 (00)	-	-
CIP	10 (27.7)	26 (72.2)	04 (50)	04 (50)	02 (50)	02 (50)	00 (00)	02 (100)	02 (10)	18 (90)
AMC	14 (38.8)	22 (61.2)	00 (00)	08 (100)	00 (00)	04 (100)	00 (00)	02 (100)	-	-
AK	32 (88.8)	04 (11.1)	02 (25)	06 (75)	04 (100)	00 (00)	02 (100)	00 (00)	16 (80)	04 (20)
CN	14 (38.8)	22 (61.2)	02 (25)	06 (75)	03 (75)	01 (25)	01 (50)	01 (50)	10 (50)	10 (50)
C	10 (27.7)	26 (72.2)	00 (00)	08 (100)	02 (50)	02 (50)	01 (50)	01 (50)	-	-
CTX	10 (27.7)	26 (72.2)	02 (25)	06 (75)	00 (00)	04 (100)	00 (00)	02 (100)	12 (60)	08 (40)
FOX	-	-	-	-	-	-	-	-	19 (95)	01 (05)
VA	-	-	-	-	-	-	-	-	19 (95)	01 (05)
FA	-	-	-	-	-	-	-	-	12 (60)	08 (40)
LZD	-	-	-	-	-	-	-	-	20 (100)	00 (00)
E	-	-	-	-	-	-	-	-	06 (30)	14 (70)
CEF	-	-	-	-	-	-	-	-	12 (60)	08 (40)
CE	-	-	-	-	-	-	-	-	06 (30)	14 (70)
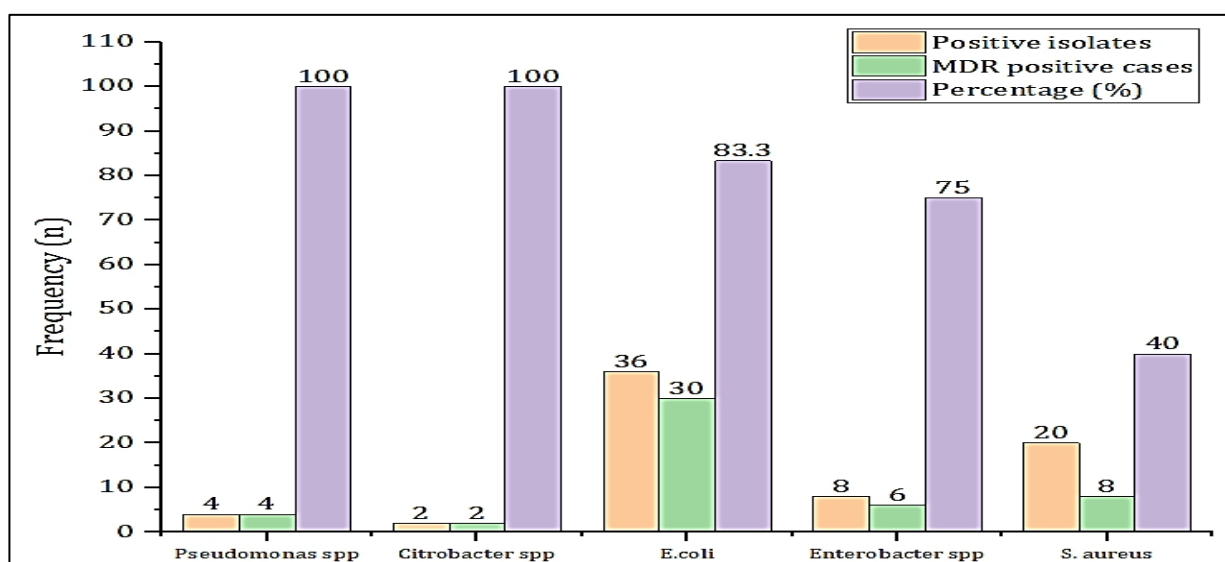
Figure 1: Prevalence of MDR Isolates in Patients with Aerobic Vaginitis

DISCUSSION:

This study was aimed to identify the MDR bacteria of aerobic vaginitis among gynae patients in the KTH, Peshawar. The current study observed the prevalence of aerobic vaginosis (35%) from different AV suspected patients. Shamim Mumtaz et al., 2008 observed the same results i.e. 38.01% of AV out of 1923 suspected patients[13]. M. Sabri A et al., 2011 have also reported the high prevalence (95.45%)[14]. The reason for our negative bacterial cultures may be the presence of another type of causative agents that might need special techniques for their detection such as viruses, Chlamydia and other causative agents. The maximum incidence rate was recorded in the age group of 21-25 years (31.4%) followed by the age group 16-20 years (25.7%). The same results were reported in a study among young females of age groups; 31-40 years (39.5%) and 41-50 years (35.8%)[13]. Another study also confirmed our results in which AV infections are found in age groups of 15-30 years and 31-40 years[15]. As lactobacilli are the normal flora of the vagina of adult females which maintain the pH of the vagina normal and also prevent the growth of the pathogenic bacteria, therefore the occurrence of infections are reduced at this age group. But the broad-spectrum antibiotics can disturb the normal flora of the vagina resulting in the growth of resistant microorganisms[14]. This study observed the high prevalence of E.coli 51.42% followed by S.aureus 28.57%, Enterobacter spp 11.42% cases and Pseudomonas spp 5.71%. The study conducted by Shamim Mumtaz et al., 2008 reported the same results; S.aureus 46%, E.coli 13.7% and P.aeruginosa 7.3%[13]. Another study conducted by M. Sabri A et al., also observed the prevalence of 25.8% in S.aureus[14]. The type of bacterial isolates and their frequency recorded in the present study were more diverse than in the study of Lakshmi et al[16,17]. Therefore, further studies to differentiate the effects of bacterial vaginosis and aerobic vaginitis on the outcome of pregnancy should be conducted. The results of current study revealed that E.coli isolates were resistant to SXT (94.4%), CIP (72.2%), CTX (72.2%), C (72.2%), AMC (61.2%), CN (61.2%) and ATM (50%) while low resistance was observed in case of MEM, IPM, SCF, TZP, AK and TGC. It was observed in a study that AV effective drugs were IPM and SCF[13]. Another study conducted by Tariq et al (2006) also reported the same results in which E.coli isolates were sensitive to tazobactam and imipenem[18]. In the current study, S.aureus showed highest resistance to CIP (90%) followed by CE (70%), E (70%), CN (50%) and CTX (40%) while 1 (5%) of each isolate was resistant to FOX (MRSA) and VA (VRSA) while some antibiotics; TGC, LZD, FOX, VA, AK, CTX, FA and CEF were effective against S.aureus isolates. The same antibiogram pattern was observed in which vancomycin was sensitive to (93.6%) isolates followed by TZP (89.13%) while showed low activity against penicillins, tetracycline (49.3%), sulphonamides (23.6%), cephalosporins (36.8%) and monobactams (19.13 %)[13]. For several years, penicillin has been used for the treatment of a variety of infections caused by S.aureus. But the organism has slowly acquired resistance against this drug. It is evident from this study where only 26.3%, 32.8% of the S.aureus showed sensitivity towards ampicillin and cephalosporins only. Mostly, S.aureus was found resistant to penicillin due to the production of ß-lactamase. Therefore, using penicillin with β-lactamase inhibitors shows much better results[19]. In current study, majority of the isolates of Pseudomonas spp, Enterobacter spp and Citrobacter spp isolates were resistant to ATM, SXT, CIP, AMC and CTX and showed low resistance to MEM, IPM, SCF, TZP, AK and TGC. The same results were also reported in which the most effective antibiotics against gram-negative rods were IPM (96%), TZP (92.1%), while low activity was observed against penicillins, tetracycline and sulfonamides[13]. MDR cases were observed in 25.71% of the isolated bacterial pathogens. This is low as compared with the study from Gondar 95%[20], and reported in Tikur Anbessa Specialized Hospital, Addis Ababa 74%[21]. The emergence of MDR cases is an alarming situation that might be due to the use of empirical treatment and lack of appropriate infection control strategies, which can cause a shift to increase prevalence of resistant organisms in the community.

CONCLUSION:

The prevalence of bacterial vaginitis was relatively high and most of the isolates were

MDR. In the current study, the majority of the gram-negative isolates were resistant to isolates were resistant to cephalosporins, tetracycline and sulfonamides while the most antibiotics; ATM, SXT, CIP, AMC and CTX while MEM, IPM, SCF, TZP, AK and TGC were effective. S.aureus isolates showed highest resistance to CIP, CE, E, CN and CTX. Therefore, comprehensive healthcare education aimed at reducing bacterial vaginitis is needed.

CONFLICT OF INTEREST: None

FUNDING SOURCES: None

REFERENCES:

1. Kaambo E, Africa C, Chambuso R, Passmore JA. Vaginal microbiomes associated with aerobic vaginitis and bacterial vaginosis. Front Public Health. 2018;6:78.
2. Han C, Li H, Han L, Wang C, Yan Y, Qi W, et al. Aerobic vaginitis in late pregnancy and outcomes of pregnancy. Eur J Clin Microbiol Infect Dis. 2019;38(2):233-9.
3. Son KA, Kim M, Kim YM, Kim SH, Choi SJ, Oh SY, et al. Prevalence of vaginal microorganisms among pregnant women according to trimester and association with preterm birth. Obstet Gynecol Sci. 2018;61(1):38-47.
4. Arooj A, Bano N, Nazir R, Chaudhri R. Comparison of combined probiotic and antibiotic therapy versus antibiotic therapy alone in treatment of bacterial vaginosis. J Soc Obstet Gynecol Pak. 2017;7(2):57-60.
5. Coleman JS, Gaydos CA. Molecular diagnosis of bacterial vaginosis: an update. J Clin Microbiol. 2018;56(9):e00342-18.
6. Zhang T, Xue Y, Yue T, Xiong L, Wang X, Wang W, et al. Characteristics of aerobic vaginitis among women in Xi'an district: a hospital-based study. BMC Women's Health. 2020;20(1):1-7.
7. Ortiz Y, García-Heredia A, Merino-Mascorro A, García S, Solís-Soto L, Heredia N. Natural and synthetic antimicrobials reduce adherence of enteroaggregative and enterohemorrhagic escherichia coli to epithelial cells. PLOS One. 2021;16(5):e0251096.
8. Hoyme UB. Efficient prevention of preterm birth as a primary political task-results of the Thuringia campaign 2017. Arch Gynecol Obstet. 2018;298:461-3.
9. Weckend MJ, Spineli LM, Grylka-Baeschlin S, Gross MM. Association between increased antenatal vaginal pH and preterm birth rate: a systematic review. J Perinat Med. 2019;47(2):142-51.
10. Suliman MA. Nasal carriage of methicillin resistant staphylococcus aureus (MRSA) among healthy primary school children in Omdurman city [dissertation]. Sudan: Sudan University of Science and Technology; 2014.
11. Alnahass R, Khaliel SA, Ellakany HF, Ibrahim MS. Comparison between bacteriological isolation and molecular detection of E. coli from chickens suffering from colibacillosis and/or diarrhea. Alexandria J Vet Sci. 2016;49(2):141-8.
12. Cusack T, Ashley E, Ling C, Rattanavong S, Roberts T, Turner P, et al. Impact of CLSI and EUCAST breakpoint discrepancies on reporting of antimicrobial susceptibility and AMR surveillance. Clin Microbiol Infect. 2019;25(7):910-1.
13. Krishnasamy L, Saikumar C, Kumaramanickavel G. Aerobic bacterial pathogens causing vaginitis in patients attending a tertiary care hospital and their antibiotic susceptibility pattern. J Pure Appl Microbiol. 2019;13(2):1169-74.
14. Ranjit E, Raghubanshi BR, Maskey S, Parajuli P. Prevalence of bacterial vaginosis and its association with risk factors among nonpregnant women: a hospital based study. Int J Microbiol. 2018;2018:1-9.
15. Kudair TT, Al-Yasiri MH, Atya AK. Characterization of pathogens community in women with vaginal infections. Univ Thi-Qar J Sci. 2021;8(1):77-81.

16. Dahal P, Jhendi S, Pun CM, Maharjan L. Assessment of risk factors and medication use for infectious vaginitis among females of reproductive age visiting maternity hospital of Pokhara, Nepal. Open Public Health J. 2017;10(1).

17. Mulu W, Yimer M, Zenebe Y, Abera B. Common causes of vaginal infections and antibiotic susceptibility of aerobic bacterial isolates in women of reproductive age attending at Felegehiwot referral Hospital, Ethiopia: a cross sectional study. BMC Women's Health. 2015;15(1):1-9.

18. Ali T, Zhang L, Shahid M, Zhang S, Liu G, Gao J, et al. ESBL-producing Escherichia coli from cows suffering mastitis in China contain clinical class 1 integrons with CTX-M linked to ISCR1. Front Microbiol. 2016;7:1931.

19. Ceballos S, Aspiroz C, Ruiz-Ripa L, Reynaga E, Azcona-Gutiérrez JM, Rezusta A, et al. Epidemiology of MRSA CC398 in hospitals located in Spanish regions with different pig-farming densities: a multicentre study. J Antimicrob Chemother. 2019;74(8):2157-61.

20. Negussie A, Worku G, Beyene E. Bacterial identification and drug susceptibility pattern of urinary tract infection in pregnant women at Karamara Hospital Jigjiga, Eastern Ethiopia. Afr J Bacteriol Res. 2018;10(2):15-22.

21. Belete MA. Bacterial profile and ESBL screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care of northeastern Ethiopia region. Infect Drug Resist. 2020;13:2579.

CONTRIBUTORS
1. **Muhammad Asghar** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
2. **Naheed** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
3. **Aakash Ahmad Khattak** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
4. **Sadiq Azam** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
5. **Noor Rehman** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
6. **Ibrar Khan** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
7. **Gul-e-Sehra** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval
8. **Anila Farid** - Concept & Design; Data Acquisition; Data Analysis/Interpretation; Drafting Manuscript; Critical Revision; Supervision; Final Approval